SEX DIFFERENCES IN SPATIAL MEMORY ABILITY:

A TEST OF THE RANGE SIZE HYPOTHESIS

IN THE ORDER CARNVIORA

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SUMMARY

Sex differences in spatial cognition have been reported for many species ranging from voles to humans. The *range size hypothesis* predicts that sex differences in spatial ability will only occur in species in which the mating system selects for differential range size. Consistent with this prediction, we observed sex differences in spatial ability in giant pandas, a promiscuous species in which males inhabit larger ranges than females, but did not observe sex differences in Asian small-clawed otters, a related monogamous species in which males and females share home ranges. Furthermore, the sex difference in giant pandas was observed during the period of male range expansion and outside female estrus, thus the potentially confounding influence of decreased female ability was avoided. Finally, all subjects in this study were raised in captivity and never actually inhabited different range sizes. Therefore these findings emphasize the importance of biological rather than experiential factors underlying sex differences in spatial cognition. These results are the first evidence of sex differences in spatial ability in the order Carnivora, and provide support for the range size hypothesis.

CHAPTER 1

INTRODUCTION

Males and females differ in many aspects of physiology, behavior, and cognition (Kimura, 2002). In many species, including humans and nonhumans, males outperform females on spatial tasks such as maze navigation (Jones, Braithwaite & Healy, 2003). This phenomenon has been investigated from proximate and ultimate perspectives, with hormones representing the most likely proximate mechanism for the difference (Spritzer, Gill, Weinberg & Galea, 2008; Williams, Barnett & Meck, 1990; Williams & Meck, 1991; Neave, 2008). The current research focused on ultimate explanations and evaluated the primary evolutionary hypotheses regarding sex differences in spatial ability [for reviews, see Ecuyer-Dab & Robert, 2004; Jones, Braithwaite & Healy, 2003; Sherry & Hampson, 1997). Various explanations for the evolution of sex differences in spatial ability have been proposed including dispersal, warfare, and division of labor (Geary, 1995; Jones et al., 2003; Silverman et al., 2000; Silverman, Choi, & Peters, 2007). However many of these hypotheses are logically flawed and/or lack generalizability, and the *range size hypothesis* and the *fertility and parental care hypothesis* provide the most plausible ultimate explanations.

The *range size hypothesis* is the best supported explanation for sex differences in spatial ability (Gaulin & Fitzgerald, 1986, 1989; Gray & Buffery, 1971). This hypothesis predicts that sex differences will occur in species in which one sex has a larger range than the other, a pattern that results from the mating system. For example, in polygynous or promiscuous mating systems, males can improve reproductive success by expanding their ranges during breeding season and remembering the locations of multiple females.

Conversely, in species in which the mating system does not select for differential range size (e.g., monogamy), there should be no obvious reproductive benefit to superior male spatial ability and no sex differences would be predicted. This hypothesis has empirical support across- and within-species.

Early work investigating the across-species predictions of the range size hypothesis was conducted with voles. As predicted, promiscuous meadow vole males have larger ranges than females and perform better on spatial tasks. Male and female monogamous prairie and pine voles have equal range sizes and performance does not differ on a spatial task (Gaulin & Fitzgerald, 1986, 1989). Similarly, polygynous rat and deer mice males have larger ranges and superior spatial performance, but there is no sex difference in monogamous desert kangaroo rats (Langley, 1994). Human beings also have a polygynous evolutionary history, so sex differences in humans can be explained by the range size hypothesis.

There is also within-species evidence for the range size hypothesis. If range expansion and improved spatial ability is a reproductive tactic, several predictions can be made. During periods of reproductive quiescence (non-breeding season/ pre-puberty), the sex difference should be minimal (Galea, Kavaliers, & Ossenkopp, 1996). As predicted, male meadow vole range size is significantly larger in breeding season than non-breeding season. There was no difference in the range size of polygynous females. For monogamous prairie voles, the range size of male and females does not change during breeding season (Gaulin & Fitzgerald, 1989). Polygynous deer mice also show differential range size only during the breeding season. Males are faster on a water maze task during breeding season, but the sex difference is not significant outside of breeding season (Galea et al., 1996). There is also support that the sex difference is not evident before sexual maturity. Juvenile meadow voles perform equally on the water maze until they are 60 days old (Galea et al., 1996). In humans, sex differences are not significant until after the age of 13 (Voyer et al., 1995). These findings are in line with the range size

hypothesis because the sex difference is linked to improved reproductive success, and would not be apparent outside of breeding season or before sexual maturity.

Alternatively, the *fertility and parental care hypothesis* proposes that sex differences do not necessarily occur because of a male advantage, but because of a female *disadvantage* during certain periods of the reproductive cycle in humans (Sherry & Hampson, 1997) and nonhumans (Ecuyer-Dab & Robert, 2004). This hypothesis proposes that female reproductive success is improved by reduced mobility during fertile periods (Sherry & Hampson, 1997). A female and her offspring will be less susceptible to injury or harm if she is less mobile and active during periods of fertility or parental care. This reduced mobility is related to impaired spatial ability. This hypothesis predicts that the sex differences result from variation in female mobility and spatial ability across the reproductive cycle. When estrogen is high performance is impaired, but when estrogen is low, performance is facilitated. Notably, this is the only hypothesis to propose that male advantage results from selection pressure acting to reduce female ability (Jones et al., 2003). Several studies find that spatial performance is impaired when estradiol is high (Galea, Kavaliers, Ossenkopp, & Hampson, 1995; Lacreuse, Verreault, & Herndon, 2001; Sherry & Hampson, 1997). This indicates that reduced mobility and spatial ability may contribute to reproductive success. However, not all studies find the expected relationship between spatial ability and estrogen level. Some studies report no changes across the menstrual cycle (Jonasson, 2005), and one study reported that performance on a spatial working memory task is worst when estrogen is low (Healy et al., 1999).

In summary, there is substantial across- and within-species support for the range size hypothesis (Jones et al., 2003); however, a more detailed analysis is necessary to differentiate between the range size hypothesis and fertility and parental care hypothesis. Both of these evolutionary mechanisms may contribute to observed sex differences; however, providing support for the range size hypothesis that is not confounded by other factors is challenging because both predict that males will outperform females, albeit for

different reasons (see Figure 1.1). Both hypotheses also predict similar patterns of within-species variation, with minimal sex differences expected outside of periods of reproductive viability (non-breeding season, before sexual maturity).

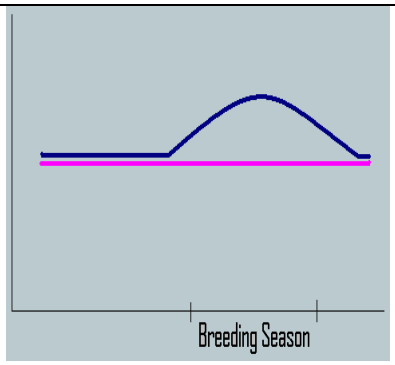
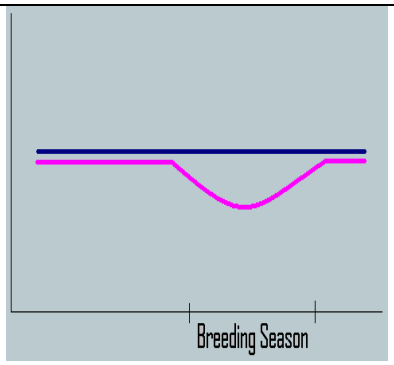
Hypothesis	**Range Size**	**Fertility and Parental Care**
Prediction	Males > Females	Males > Females
Why?	Males better during breeding season	Females worse during breeding season
Predicted Performance on Spatial Task Female Male		

Figure 1.1. Predictions of Range Size and Fertility and Parental Care Hypothesis

Little work has focused on distinguishing between these evolutionary hypotheses, yet it is critical to test separate predictions of each in order to eliminate and/or refine existing hypotheses (Jones et al., 2003). One way to provide unambiguous support for the range size hypothesis is to demonstrate the presence of a relationship between sex differences and range size *outside* of the breeding season (Jones et al., 2003). However, the period of male range expansion and female estrus overlap for many species; thus, a

rigorous test of the hypothesis would require the use of a species in which these processes are temporally distinct. Advances in evolutionary theory can be achieved by testing species characterized by this pattern.

Measuring Spatial Ability

In order to investigate evolutionary hypotheses, it is critical to determine the most appropriate task for measuring spatial ability in a given species. Spatial cognition is a multi-dimensional concept, including areas such as navigation, place learning and spatial memory. These abilities are critical for survival because an individual can find and later remember the location of resources such as food, mates, and avoid predators (Perdue, Snyder, Pratte, Marr, & Maple, 2009). At least two aspects of spatial memory can be measured: spatial working memory and spatial reference memory (Olten & Papas, 1979). Reference memory pertains to information that is constant across sessions, and working memory pertains to information that changes within a session (Olten & Papas, 1979; Shettleworth, 1998). There are neurological, hormonal, and behavioral studies supporting the distinction between working and reference memory (Lacreuse et al., 2005; Olten & Papas, 1979; Spritzer, Gill, Weinberg, & Galea, 2008). Generally, research shows that the magnitude of sex differences in spatial memory is greater on working memory tasks.

A number of techniques have been developed to assess spatial working and reference memory. Typically, they require a subject to remember a goal location or avoid an aversive location (Astur, Tropp, Sava, Constable, & Markus, 2004). Examples include the starburst maze, spiral Battig maze, radial mazes, open-area mazes, runway mazes, water mazes, oasis maze (dry analog of water maze), atlantis platform, and annular mazes (Astur et al., 2004; Clark & Martin, 2005). The most commonly used techniques in the animal literature are the Morris water maze and the radial arm maze (Astur et al., 2004). In the Morris water maze task, rats are placed in a pool of opaque water and must learn the location of a submerged platform (Morris, Garrud, Rawlins, & O'Keefe, 1982). In the

final session, the "probe trial," the platform is removed and the amount of time in the correct quadrant of the maze is recorded. The initial task assessed reference memory, but subsequent adaptations of the water maze allow for tests of working and reference memory. In the working memory component, subjects are trained on a delayed match-to-place task. On an exposure trial, the subject finds the platform in a particular location. After a delay, the subject must find the platform in the same location on the retention trial. The next day, a new location is used (Sandstrom, Kim, & Wasserman, 2006). The Morris water maze may not be ideal for all species because it relies on aversive motivation, and may be physically demanding and stressful for subjects (Astur et al., 2004). An alternative test of spatial memory is the radial arm maze.

The initial radial arm maze design tested spatial working memory. Rats were placed on a center platform and then allowed to travel down any of the eight alleys to retrieve a food item. Subjects were highly accurate at retrieving all food items without re-visiting alleys (Olten & Samuelson, 1976). A critical development in the radial arm maze paradigm is the technique of baiting only half of the arms. This procedure allows for the separate measurement of working and reference memory errors (Olten & Papas, 1979). The baited set of arms comprises the working memory component of the task. An optimal strategy on the working memory component of the task would be to visit an arm once and then never return in a session. The unbaited set of arms comprises the reference memory component of the task. An optimal strategy on the reference memory component of the task would be to never visit those locations.

The standard radial arm maze can accommodate a wide variety of species, such as rats, rabbits, hedgehogs, guinea pigs, crows and chickens (Lipp et al. 2001). Analogs of the radial arm maze have also been developed for testing with other species. For example, birds are often tested in an open field analog of the radial arm maze. Feeders are placed equidistantly around an open space and can be accessed without traveling through arms (Lipp et al. 2001). Other mammal species have been tested with similar analogs, such as

simulated foraging tasks. Foraging tasks require a subject to remember the location of hidden food items. Some tasks require subjects to return to a previously visited location (win-stay), and other tasks require subjects to avoid previously visited locations and search locations that did not previously contain food (win-shift); (Bicca-Marques, 2005). Simulated foraging tasks may require a win-stay strategy, win-shift strategy, or both. Simulated foraging tasks have been used in a variety of domesticated (sheep: Dumont and Petit, 1998), wild (emperor tamarins, saddle-back tamarins, titi monkeys: Bicca-Marques, 2005) and captive animals (gorillas, orangutans, marmosets, yellow-nosed monkeys: MacDonald, 1994; MacDonald & Agnes, 1999; MacDonald, Pang, & Gibeault, 1994; MacDonald & Wilkie, 1990). In these tasks, feeders or specific locations were baited with food. On subsequent visits to the enclosure, subjects had to visit the previously baited location (win-stay) or previously un-baited location (win-shift) to find food.

Tarou (2003) tested spatial learning in giant pandas and spectacled bears using a simulated foraging task. Eight feeders were mounted around an enclosure and food items were placed inside of the feeders. Subjects were tested with a spatial task (analogous to the win-stay task). Four of the feeders were baited for each trial. Correct visits were those made to baited feeders and incorrect visits were those made to unbaited feeders. Five of the subjects were able to use spatial cues to locate food within 30 trials (criterion required subjects to make 3 correct choices in the first 4 visits on four out of five sessions). To determine whether the subjects were relying on visual and olfactory cues, rather than spatial cues, a reversal task was included. In the reversal task, the originally unbaited sites were baited with food. Initially, subjects that had learned the spatial task visited the originally baited sites (even though they no longer contained food). This disturbance in performance suggests that the subjects were not using olfaction or vision to locate the baited feeders (Tarou, 2003).

Foraging tasks are a useful adaptation of the radial arm maze that provide a great deal of information on spatial memory, and can be easily implemented in captive or wild settings.

Sex Differences in Spatial Ability

For many years, research revealed sex differences in both human and nonhuman animals, but these studies were carried out using very different techniques. Humans were traditionally tested "with" mazes or pen-and-paper tasks, while animals were traditionally tested "in" mazes (Washburn & Astur, 2003). Many of the standard animal procedures, including the Morris water maze and radial arm maze, have been adapted for virtual environment in humans (Astur et al., 2004). Thus, similar experiments can be used to identify sex differences across species. Examples of the male advantage in spatial cognition range from horses to cuttlefish (Jozet-Alves, Moderan, & Dickel, 2008; Murphy, Waldmann, & Arkins, 2004), but the majority of research has been conducted in rodents and primates.

Order Rodentia

Regardless of rearing conditions and number of available cues, male rats are more accurate and make fewer errors than females on the radial arm maze (Seymoure, Dou, & Juraska, 1996). Similarly, male mice make fewer errors on working and reference memory components of the radial arm maze (Gresack & Frick, 2003). However, there are some studies that do not report a sex difference (e.g., Healy, Braham, & Braithwaite, 1999). A recent meta-analysis reviewed and examined sex differences in rat and mouse spatial memory (Jonasson, 2005). Studies were selected that used the radial arm maze or Morris water maze. Findings indicated a large and robust male advantage on the water maze and the radial arm maze. Jonasson (2005) concluded that any apparent inconsistency in the literature regarding sex differences is probably due to small sample

sizes and insufficient power. The largest effect size was found for tasks using the radial arm maze design with both a reference and working memory component. The pooled male advantage on the working memory component yielded a large effect size (*d_{avg}= .85), as did the reference memory component (*d_{avg} = .61). These values indicate that the male average score is .85 standard deviations (s.d.) higher than the female score on the working memory component, and .61 s.d. higher on the reference component. In general, there is a robust advantage in male rat and mouse spatial memory. In addition to rats and mice, a concurrent line of research has confirmed sex differences in spatial cognition in meadow voles (Gaulin & Fitzgerald, 1986, 1989; Kavaliers, Ossenkopp, Galea, & Kolb, 1998).

Order Primate

Human spatial memory has been tested using a wide variety of techniques. As previously mentioned, it was assumed that pen-and-paper tasks in humans and mazes in animals were tapping into the same ability, but this remained largely untested until recently (Moffat, Hampson, & Hatzipantelis, 1998). There is support that this assumption is correct because performance on standard psychometric tests does correlate with navigation on virtual mazes. For example, mental rotation ability is highly correlated with performance on virtual maze navigation (Moffat et al., 1998). Some have speculated that navigating around an object and mentally rotating the object involve a similar process: perceptual constancy (Silverman et al., 2000). This speculation provides a rationale for the robust male advantage reported in mental rotation tasks. A recent meta-analysis reported a half standard deviation male advantage on mental rotation (Voyer, Voyer, & Bryden, 1995). Although psychometric tasks correlate with maze navigation, the latter may be a more ecologically valid technique for comparison to the animal literature (Moffat et al., 1998).

Efforts to immerse humans "in mazes" include real-world navigation tasks, virtual reality, and virtual environments (Kelly & Gibson, 2007). Real-world navigation tasks lack strict control and virtual reality is expensive and may result in cybersickness. Virtual environments allow subjects to navigate through a scene displayed on a desktop computer or projector. Virtual environments allow for humans to be tested in a dynamic environment while still allowing for experimental control (Maguire, Burgess, & O'Keefe, 1999). Virtual versions of the Morris water maze and radial arm maze are particularly useful for comparison to the animal literature because these are the most commonly used tests of spatial memory.

On the virtual Morris water maze, multiple studies have found that males were significantly faster and more accurate at locating the platform, and spent more time in the appropriate quadrant during probe trials (Astur et al., 2004; Mueller, Jackson, & Skelton, 2008). On a version of the task in which the platform is visible, males and female performance did not differ (Astur, Ortiz, & Sutherland, 1998). These findings suggest that the sex difference is not due to motivational, motor or sensory demands. Furthermore, all of these studies controlled for previous experience with computer games and technology. Males were faster than females on a virtual radial arm maze task, but committed a similar number of working and reference memory errors (Astur et al., 2004). The virtual radial arm maze may differ from the water maze for several reasons: 1) the number of possible routes is limited and discrete 2) a subject only needs to remember a single cue associated with an arm 3) only direction is relevant, while the tasks such as the water maze requires distance and direction information (Astur et al., 2004). The use of a simulated foraging version of the radial arm task would eliminate those possible differences.

Studies of non-human primates also report a male advantage, although it varies across the lifespan. Young rhesus macaques outperformed females on the spatial Delayed Recognition Span Task (DRST), but there was no sex difference for older subjects

(Lacreuse, Herndon, Killiany, Rosene, & Moss, 1999). There were no sex differences on any of the non-spatial components of the cognitive test battery. Another study confirmed the male advantage in young, but not old rhesus macaques using a larger sample of 90 individuals. The follow-up study reported that the sex difference was specific to working memory, and there was not a significant difference in male and female performance on a reference memory version of the task (Lacreuse et al., 2005). In summary, there is a male advantage in spatial memory among primates and rodents which appears to be most evident when using working memory tasks.

Though valuable insights have been gained from work testing rodents and primates, further advances in evolutionary theory can be accomplished by testing a broader range of species. In particular, testing a species in which male range expansion and female estrus are temporally distinct will allow for a clear test of the range size hypothesis. Sex differences in spatial ability have never been tested in the order Carnivora despite the potential contributions that could be achieved by doing so.

A Test of Evolutionary Hypotheses in the Order Carnivora

Intra-sexual territoriality is a common sociobiological organization in the order Carnivora (Johnson, Macdonald, & Dickman, 2000). The classic intra-sexual territory model involves males maintaining larger ranges that encompass multiple female ranges. However, males typically exhibit distinct ranging patterns across breeding and non-breeding seasons (Sandell, 1989). During non-breeding season, male range size is determined by the distribution of food or resources. During breeding season, male range size increases to include as many females as possible. Across carnivore species, male ranges are 2.5 times larger than female range, even though metabolic needs would only require a range 1.2 times larger. Thus, for the majority of species in the order Carnivora, male ranges are determined by factors other than food; specifically, the distribution of females (Sandell, 1989). However, approximately 7% of species in the order are

characterized by a monogamous mating system (Dalerum, 2007) and equivalent range sizes.

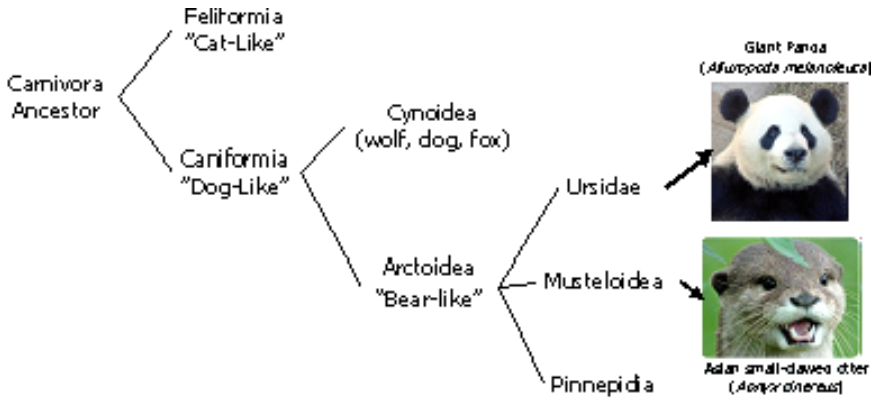


Figure 1.2. Phylogeny of the order Carnivora

According to the range size hypothesis, sex differences in spatial ability should be observed in a promiscuous species with differential range sizes, but no sex differences would be expected in a monogamous species with equal home range sizes. This research is the first empirical investigation of the range size hypothesis in the order Carnivora. I predicted that promiscuous male giant pandas would outperform females on a spatial memory task, but there would be no sex differences in the performance of monogamous Asian small-clawed otters. Giant pandas and Asian small-clawed otters are both on the Arctoidea branch of the suborder Caniformia within the order Carnivora (see Figure 1.2), thus testing with these species will allow for a valid comparison to be made. Furthermore, male giant panda range expansion begins several months prior to female estrus (Schaller, Jinchu, Wenshi, & Jing, 1985). Testing for sex differences in spatial

ability during the period of male range expansion in giant pandas, but prior to female estrus, allows for a test of the range size hypothesis while controlling for the potential effect of decreased female spatial ability during estrus.

CHAPTER 2

SEX DIFFERENCES IN GIANT PANDA SPATIAL MEMORY

Giant pandas provide an excellent model for testing the range size hypothesis. In the wild, giant pandas are solitary and social interactions are primarily limited to the mother-infant relationship and breeding encounters. Giant pandas have a promiscuous mating system in which both males and females mate with multiple partners during the breeding season (Schaller et al., 1985). This mating system has resulted in differential range size. Male giant pandas have larger ranges that overlap with the smaller, more concentrated ranges of multiple females (i.e., intra-sexual territory). The average male range is 6.7 km^2 and the average female ranges is 4.5 km^2 (Schaller et al., 1985). Male and female range size also differs substantially when considering the actual "use of space" rather than total range size. Females have smaller, tightly defended core areas (30-40 hectares) in which they spend the majority of their time. Males have large, overlapping ranges and visit the space more often (Schaller et al., 1985).

Giant panda females typically undergo one estrous period per year (Schaller et al., 1985), generally occurring in early spring. The total estrous period lasts 12-25 days, but peak receptivity is only 2-5 days. Corresponding hormonal and behavioral changes in the female are largely limited to that period (Schaller et al., 1985; Lindburg, Czekala & Swaisgood, 2001). In contrast, male hormonal and behavioral changes begin several months before peak female estrus (Schaller et al., 1985). Beginning in November, males start to roam persistently and testosterone levels are elevated at least three months prior to estrus (Schaller et al., 1985; Tsutsui et al., 2006). Males monitor the location of other males, females, and communal scent-marking locations during this period. By testing for sex differences in spatial ability during the period of male range expansion, but prior to

female estrus, we can provide support for the range size hypothesis while controlling for the potential effect of decreased female spatial ability during estrus (see Figure 2.1).

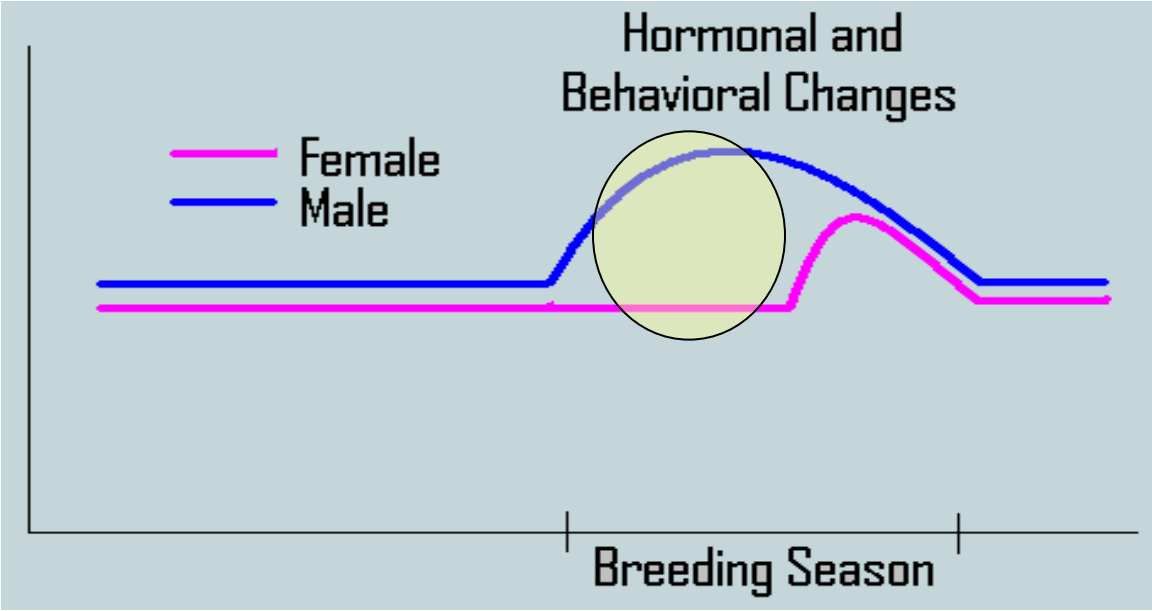


Figure 2.1. Schematic of giant panda breeding season.

The highlighted section (Figure 2.1) depicts the time period of testing that includes male range expansion that occurs approximately beginning in November, but prior to female estrus in early spring. If range expansion results in differential spatial ability, the sex difference should be evident in the period leading up to breeding season (i.e., range size hypothesis). However, if reduced female spatial ability results in the sex difference (i.e., fertility and parental care hypothesis), there will be no sex difference during this period. As previously discussed, the period of male range expansion and female estrus overlap

for most species. This distinct pattern in the giant panda allows for a unique test of sex differences in spatial ability.

Giant panda spatial memory has been previously tested (Perdue et al., 2009), including a study which used simulated foraging task (Tarou, 2003; Tarou, Snyder, & Maple, 2004). However, these data were collected outside of breeding season or with sexually immature subjects, so a thorough investigation of sex differences was not possible. Nonetheless, results of both studies were in accordance with the predictions of the current research. Tarou (2003) found that male giant pandas committed fewer working memory errors than females on a spatial task that is identical to the one used in the current study. During the first five sessions of the spatial task, females revisited (i.e., committed a working memory error) an average of 4.75 feeders per session while males only made 0.5 revisits per session. Perdue et al. (2009) found that one male performed better on a spatial memory recall task than a female. The male subject recalled the spatial location more often than the female on trials with 3-, 5-, 6- and 10-second delays between the presentation of the correct location and the response. Based on the range size hypothesis and performance on previous tasks, I predicted that male giant pandas would outperform females on a spatial task.

Method

Subjects

We tested 17 giant pandas (8 male, 9 female) housed at the Chengdu Research Base and the Chengdu Zoo in Sichuan Province, China (see Table 2.1).

Table 2.1. Giant panda subject names, sex, and date of birth

Name	Studbook Number	Sex	Date of Birth
Qi Zhen	490	F	9/4/1999
Shu Qing	480	F	8/3/1999
Qing He	537	F	9/24/2001
Cheng Gong	522	F	9/11/2000
Cheng Ji	523	F	9/12/2000
Jiao Zi	425	F	8/21/1995
Mao Mao (Fu Wa)	570	F	9/6/2003
Yuan Yuan	561	F	8/1/2003
Si Yuan	593	F	10/22/2004
Bing Dian	520	M	9/1/2000
Ya Guang	530	M	8/20/2001
Kebi	386	M	7/26/1992
Lin Lin	455	M	8/2/1997
Xiao Ping Ping[a]	342	M	9/1987
Yong Yong	584	M	8/26/2004
Long Bang	573	M	9/8/2003
Qiu Bing	574	M	9/8/2003

[a]Subject was born in the wild and brought into captivity at approximately six months old

Subjects were tested from November 2009 - February 2010, a period which corresponds to male range expansion and testosterone elevation (Schaller et al., 1985; Tsutsui et al., 2006), but no behavioral or physiological indicators of estrus in females were observed (personal observation).

Apparatus

Feeders were constructed from a wooden frame with an opaque lid. Feeders were designed so that depleted feeders were not visually apparent or distinct from other feeders (Tarou, 2003) (Figure 2.2). The stainless steel lid was attached with a spring-loaded hinge

so that the lid remained closed unless being physically held open. Thus, a subject interacting with a feeder did not alter its appearance (i.e., after a subject visits a feeder, its appearance did not change and visual cues could not be used to guide behavior).

Figure 2.2. Giant panda feeder

Giant pandas could open the lid by using their muzzle or paw, and retrieve food item directly with mouth or remove using paw and then consume (Figure 2.3).

Figure 2.3. Giant pandas could open the lid of the feeder with muzzle or paw.

Eight feeders were arranged in a circular pattern in an outdoor enclosure (Figure 2.4). Feeders were cleaned between sessions.

Figure 2.4. Feeders were installed equidistantly in a circular pattern.

Procedure

One apple was used for each session. Whole apples were cut into four even pieces. Prior to each session, all feeders were rubbed with the scent of the apple so that the baited feeders could not be identified by olfactory cues. Then, a quarter of an apple was placed inside a feeder. The same four feeders were baited with the food item, and the other four were left empty across all sessions. Subjects were allowed to freely explore the feeders. Any contact with a feeder was recorded as a visit. The order, location, and time of each visit were recorded for each session (Appendix A). Sessions lasted for 20 minutes. Nine test sessions were conducted for each giant panda.

The day after the test sessions, a "probe trial" was conducted in which all feeders were rubbed with the scent of the food item, but none were baited with food. The probe trial further tested the possibility that subjects were using extraneous cues (e.g., scent) rather than spatial cues to find food in the previous task. Only four visits were needed to recover all food items, so the proportion of the first four responses to correct locations was determined. If this performance fell below chance when food was absent, subjects may have been relying on cues from the food itself. However, if performance remained above chance on the "probe trial," then spatial location was the relevant information used to solve the task.

Behavioral data were also collected. For each subject, at least ten 30-minute behavioral observation sessions were conducted. Sessions were balanced between morning and afternoon for each subject. A general giant panda ethogram was used (Appendix B). A combination of all-occurrence, instantaneous (1-minute samples) and one-zero sampling was used (Appendix C). Visits to feeders during behavioral observations were recorded, including whether the visit was to a correct ("depleted") or incorrect ("never baited") location. These observations took place after the spatial task and all food items have been retrieved.

Data Analysis

Several measures of performance on the spatial memory task were recorded (See Table 2.2). For each of these measures, test trials were summarized into 3-session intervals, resulting in three intervals.

Table 2.2. Spatial task measures and definitions

Measure	Definition
Total Number of Visits	Number of visits within a session (within 20 minutes of the first visit)
# Correct in First Four Visits	Number of visits to baited location in first four visits. Only four visits are needed to locate all food items.
Reference Memory Error	Visit to a location that is never baited with food.
Working Memory Error	Re-visit to a location that has already been visited in that session.
Working Memory Error – Depleted Feeder	Re-visit to a correct location (baited with food) that has already been visited in that session.
Working Memory Error – Never Baited Feeder	Re-visit to an incorrect location (never baited with food) that has already been visited in that session.
Total Number of Visits	Number of visits within a session (within 20 minutes of the first visit)

Did subjects learn the task?

Performance on the probe trial was compared to chance performance using a one-sample t-test ($\alpha = .05$). No food was present during the probe trial. If subjects were responding based on spatial location, and not on visual or olfactory cues, performance on the probe trial should be above chance.

I specifically examined working memory errors (i.e., re-visits) to determine if there was a pattern underlying which locations subjects re-visited during a session. The number of re-visits to correct (i.e., depleted feeders) was compared to the number of re-

visits to incorrect locations (i.e., feeders that were never baited) using a nonparametric paired samples test, the Wilcoxon signed ranks test ($\alpha = .05$). If subjects were learning the spatial location of food items, it would be expected that subjects return to depleted feeders more often than never baited feeders.

I also tested whether performance on all measures improved across sessions using a nonparametric repeated measures test, Friedman's test ($\alpha = .05$).

Sex differences?

For each interval, the performance of males and females was compared using a Mann-Whitney U test for each measure ($\alpha = .05$). The *a priori* hypothesis was that males would outperform females, therefore a one-tailed test was used. To analyze the behavioral data, a Mann Whitney U test was used to compare average male and female durations and/or rates of various behaviors (see Table 2.4). For locomotion and scent marking, the a priori hypothesis was that males would perform these behaviors more often, therefore a one-tailed test was used. Two-tailed tests were used for all other comparisons.

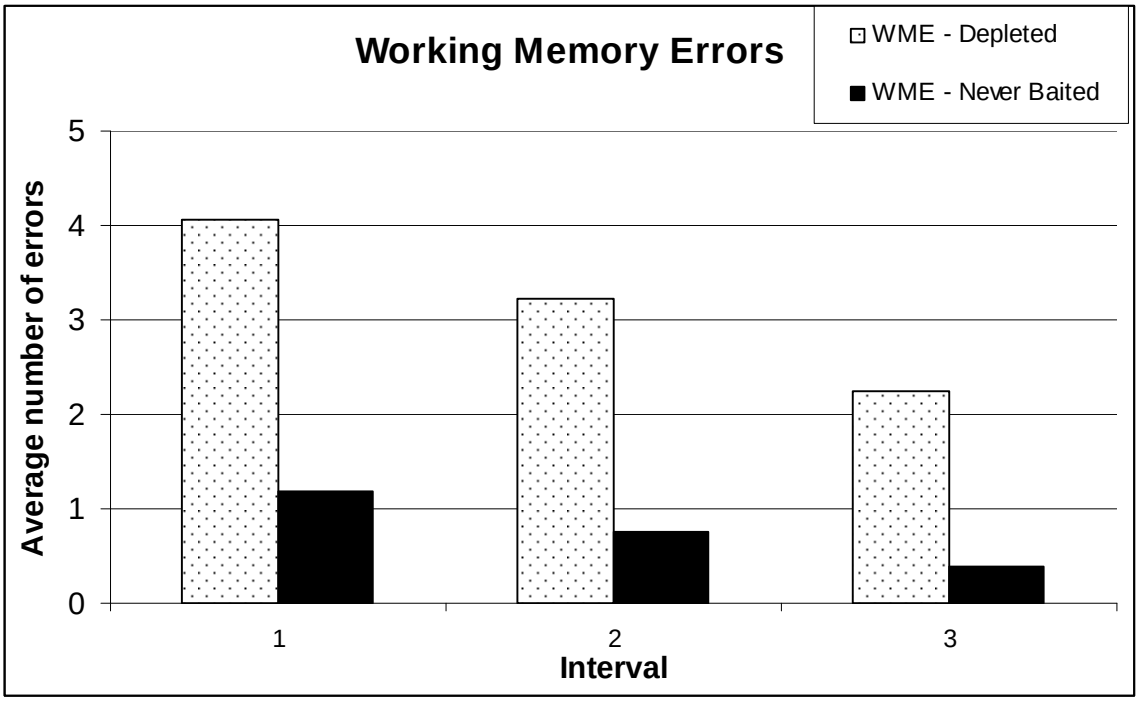
Results

Did subjects learn the task?

On the probe trial, giant pandas ($t = 4.197$, $df = 16$, $p < 0.05$) were significantly more likely than chance to visit to correct locations (i.e., baited on previous trials) in the first four visits, even though no food was present. Subjects re-visited depleted feeders significantly more often than never baited feeders in all three intervals (see Table 2.3 and Figure 2.5).

Table 2.3. Working memory errors to depleted or never baited feeders.

Interval	Wilcoxon Signed Ranks test
1	**Z = -3.524**
	p = .000
2	**Z = -3.628**
	p = .000
3	**Z = -3.305**
	p = .001



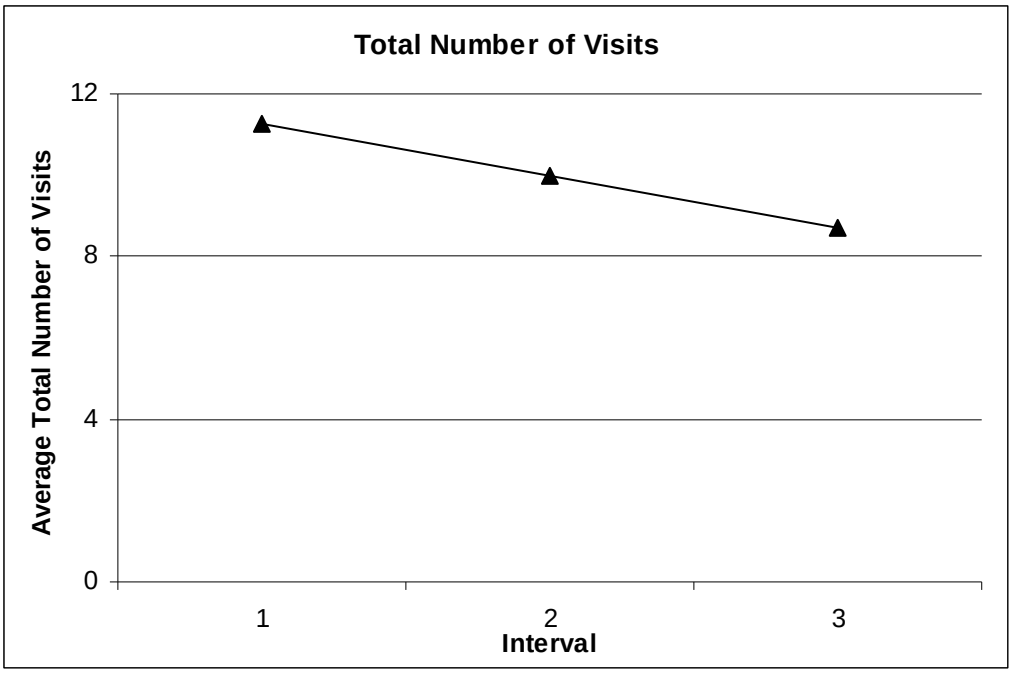
*Significant difference in all intervals

Figure 2.5. Difference in working memory errors to depleted or never baited feeders.

There was a significant increase in the number correct in the first four visits across intervals, and a significant decrease in the total number of working memory errors and the number of working memory errors to depleted feeders (see Table 2.4 and Figure 2.6 – 2.11).

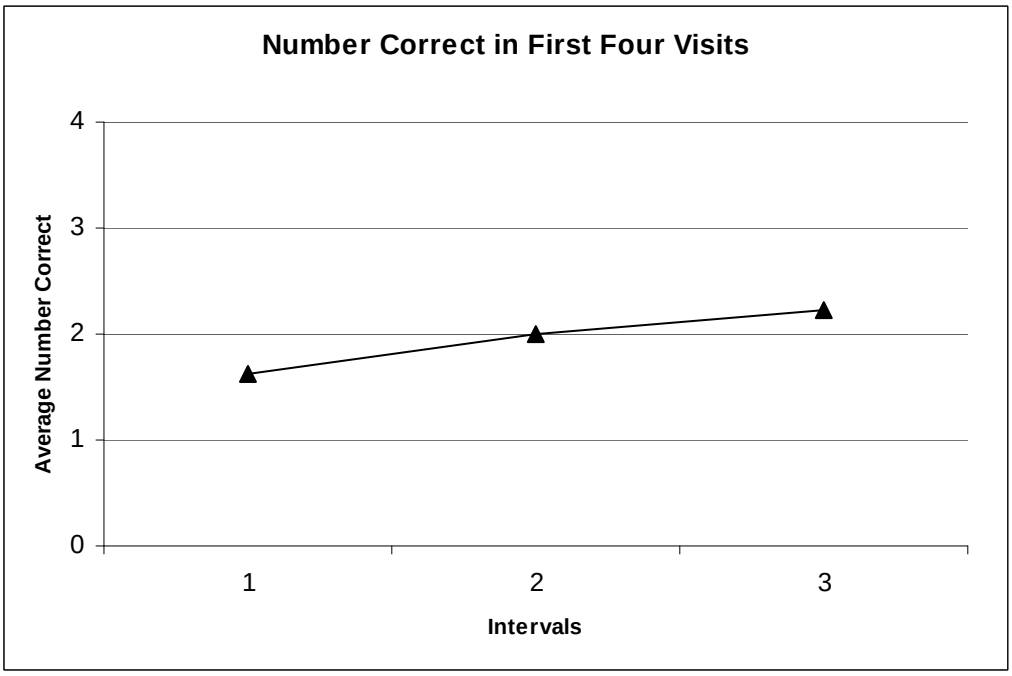
Table 2.4. Change across intervals.

Measure	Friedman's test
Total Number of Visits	$X^2 = 4.364$ $p = .113$
# Correct in First Four Visits	$\mathbf{X^2 = 18.421}$ $\mathbf{p = .000}$
Reference Memory Error	$X^2 = .286$ $p = .867$
Working Memory Error	$\mathbf{X^2 = 15.364}$ $\mathbf{p = .000}$
Working Memory Error – Depleted Feeder	$\mathbf{X^2 = 16.369}$ $\mathbf{p = .000}$
Working Memory Error – Never Baited Feeder	$X^2 = 3.875$ $p = .144$



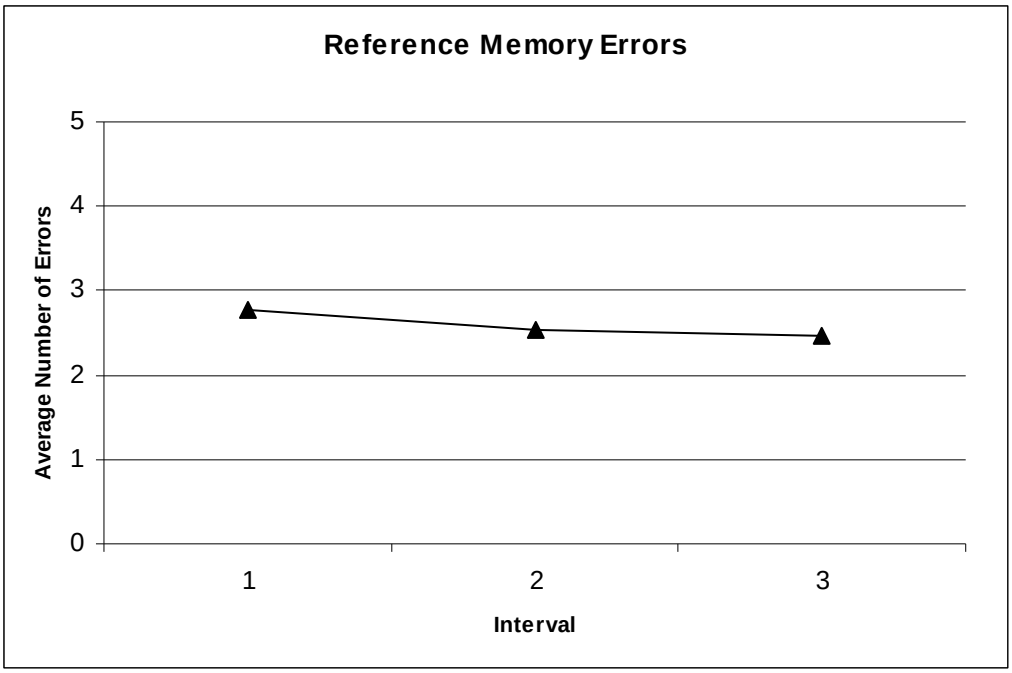
*No significant change

Figure 2.6. Total number of responses across intervals



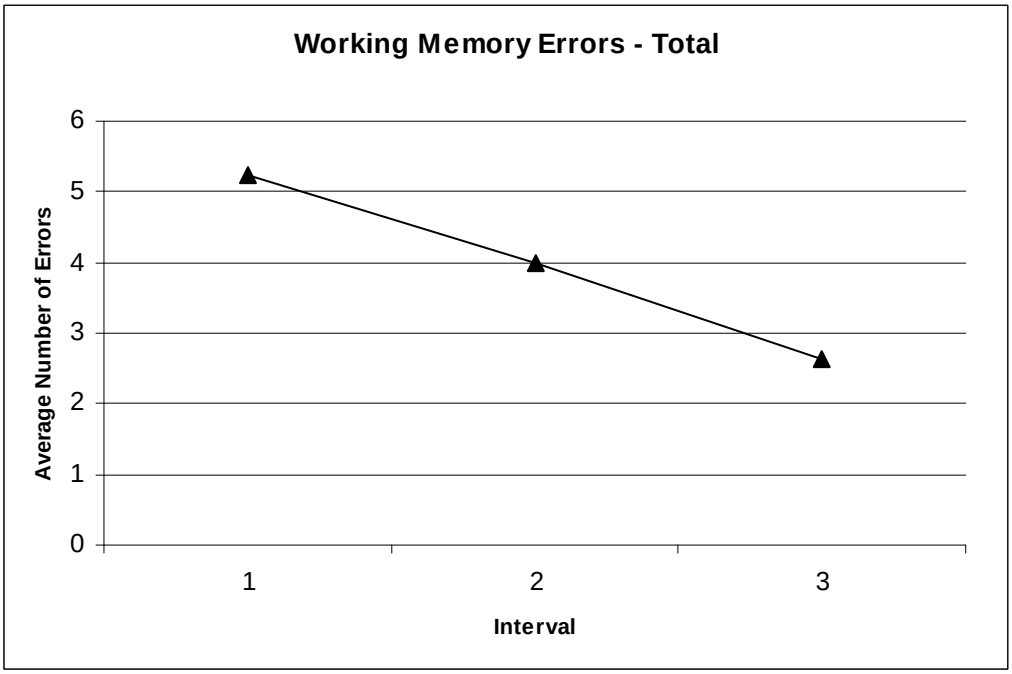
*significant increase

Figure 2.7. Number correct in first four visits across intervals



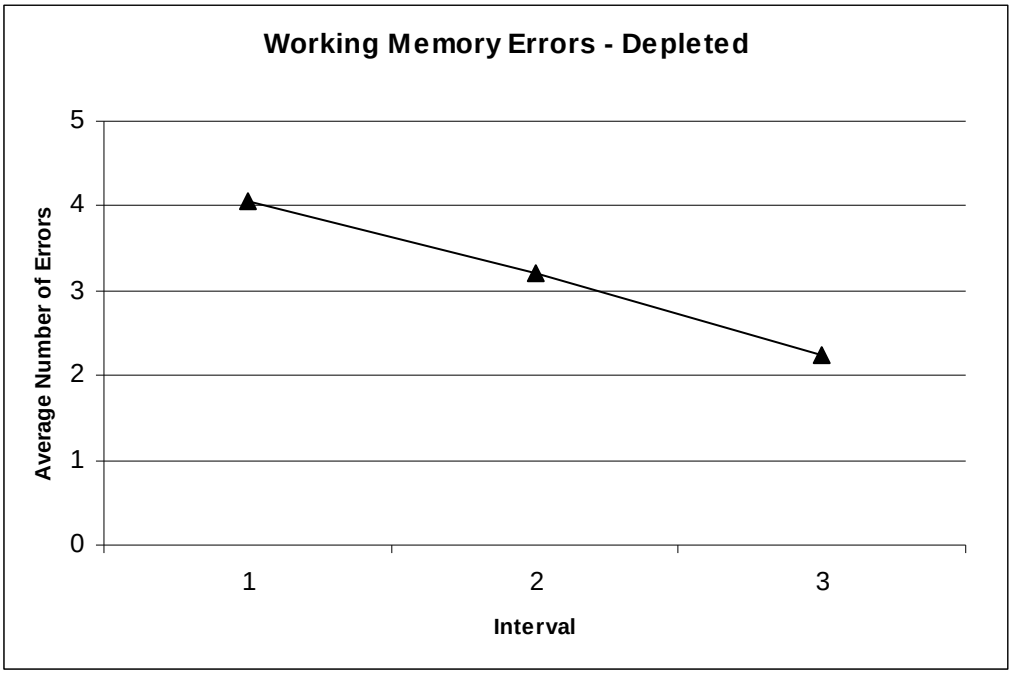
*No significant change

Figure 2.8. Reference memory errors across intervals



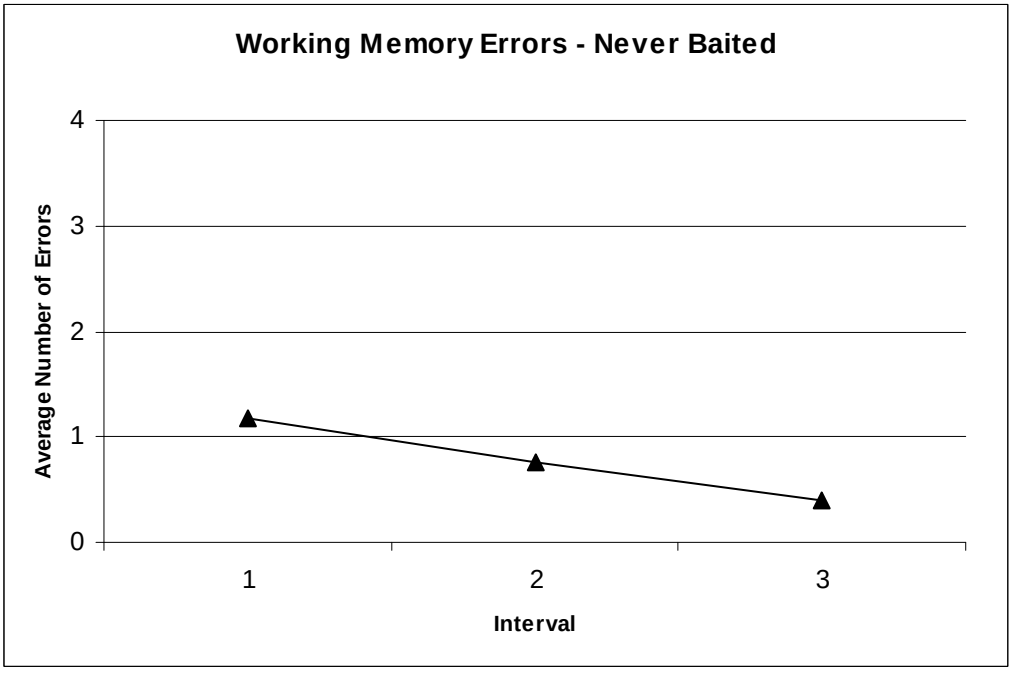
*significant decrease

Figure 2.9. Working memory errors (total) across intervals



*significant decrease

Figure 2.10. Working memory errors (depleted) across intervals



*No significant change

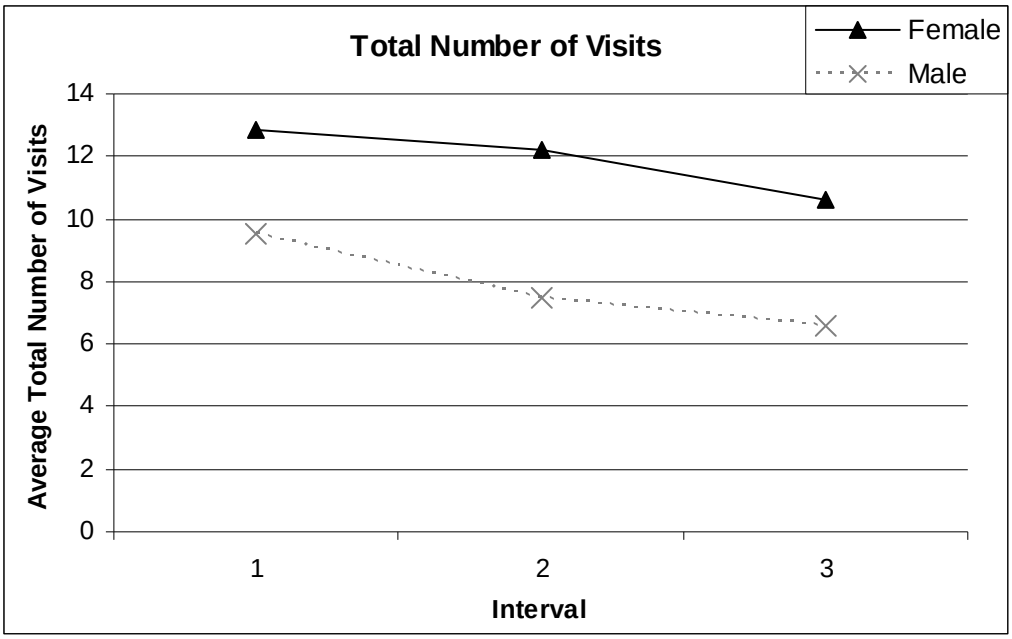
Figure 2.11. Working memory errors (never baited) across intervals (ns).

Sex differences?

There were many significant differences between males and females on the spatial task (see Table 2.5 and Figure 2.12 – 2.17). Overall, differences were found for the total number of visits, reference memory errors, and working memory errors, with males outperforming females on all measures in which significant differences were observed (see Table 2.5 and Figure 2.12 – 2.17).

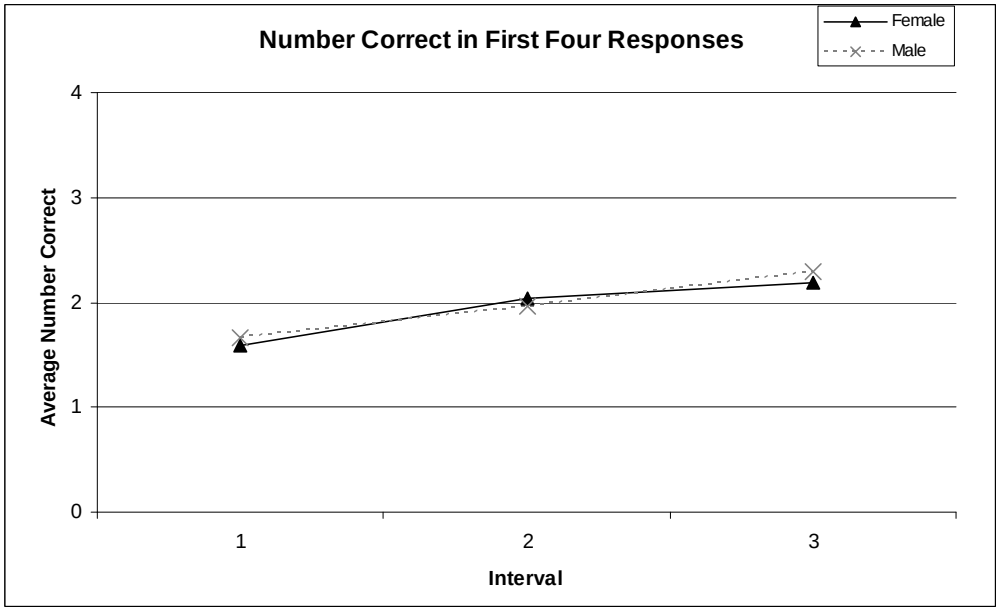
Table 2.5. Mann-Whitney U statistic and p-value (one-tailed) for all measures

Measure	Statistic	Interval		
		1	2	3
Total # of Visits	U	20.0	**7.5**	**11.5**
	p-value	0.07	**0.002**	**0.008**
# Correct in First Four Visits	U	32.0	33.0	29.5
	p-value	0.372	0.408	0.271
Reference Memory Error	U	32.5	**18.5**	**17.5**
	p-value	0.372	**0.047**	**0.037**
Working Memory Error	U	**13.5**	**9.5**	**9.0**
	p-value	**0.014**	**0.004**	**0.004**
Working Memory Error – Depleted Feeder	U	**12.0**	**10.0**	**8.0**
	p-value	**0.011**	**0.006**	**0.003**
Working Memory Error – Never Baited Feeder	U	25.0	**12.0**	**16.0**
	p-value	0.161	**0.011**	**0.030**



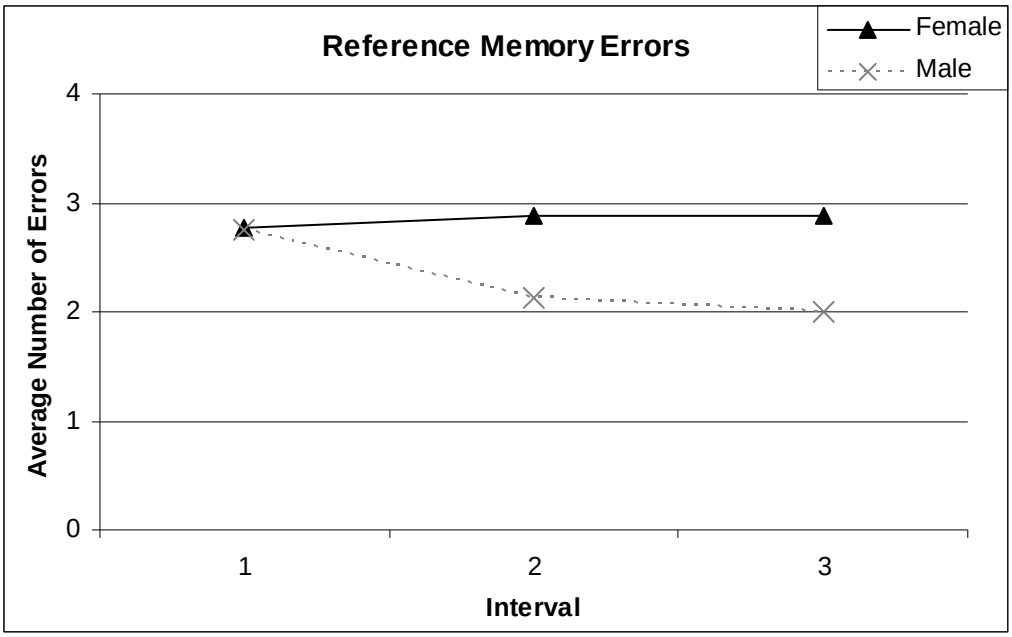
*significant difference in interval 2 & 3

Figure 2.12. Sex differences in total number of visits



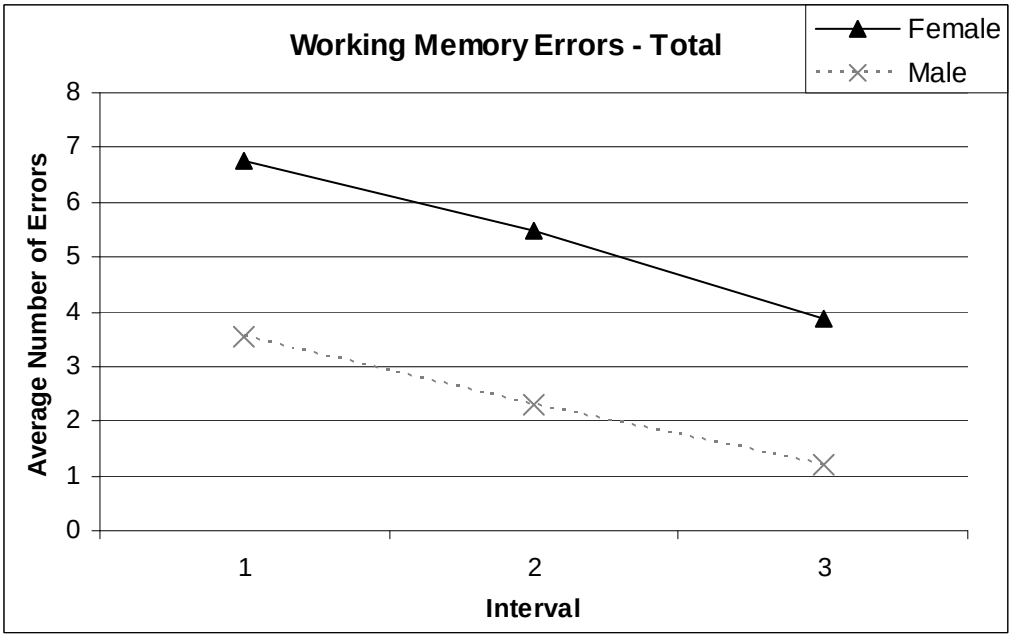
*No significant differences

Figure 2.13. Sex differences in number correct in the first four visits



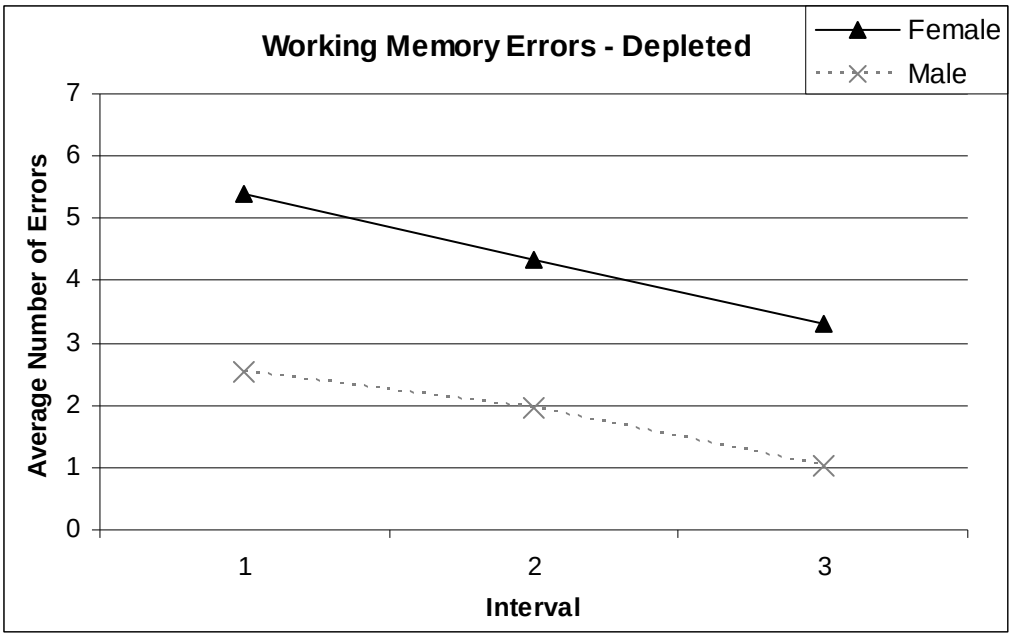
*significant difference in interval 2 & 3

Figure 2.14. Sex differences in reference memory errors



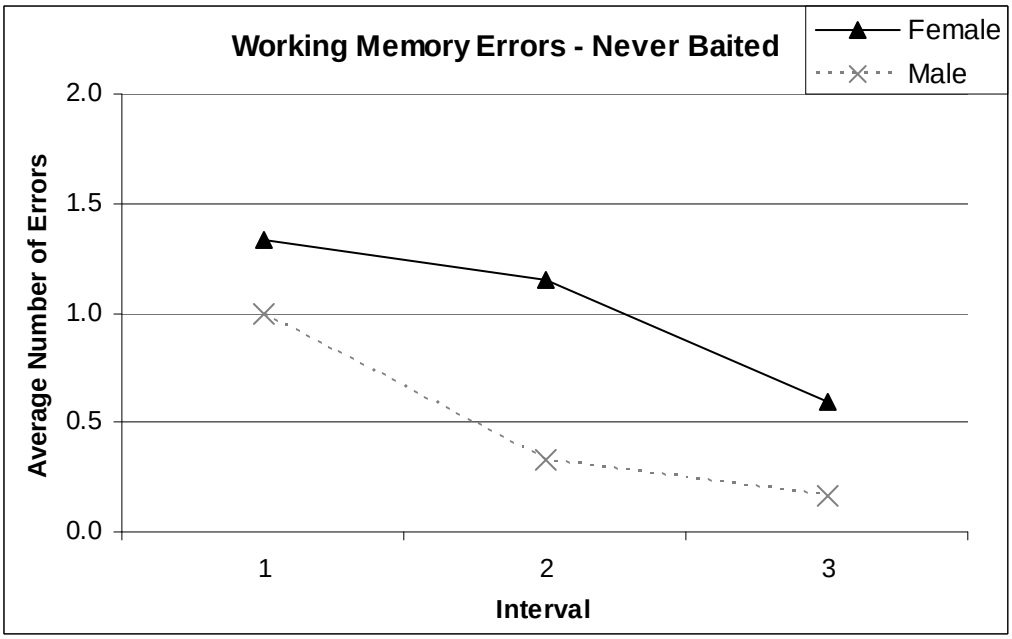
*significant difference in interval 1, 2 & 3

Figure 2.15. Sex differences in working memory errors (total)



*significant difference in interval 1, 2 & 3

Figure 2.16. Sex differences in working memory errors (depleted)



*significant difference in interval 2 & 3

Figure 2.17. Sex differences in working memory errors (never baited)

There were also a number of significant sex differences in behavior (see Table 2.6 and Figures 2.18 - 2.19).

Table 2.6. Sex differences in behavioral measures.

Behavior	Type of Measurement	Mann-Whitney U
Door Directed	Duration	U = 35.0
		p = .963
Feed Bamboo	Duration	**U = 13.0**[a]
		***p* = .027**
Feed Other	Duration	U = 25.0
		p = .321
Drink	Duration	U = 16.5
		p = .059
Locomote*	Duration	**U = 16.0**[a]
		***p* = .03**
Maintenance	Duration	U = 32.0
		p = .743
Rest	Duration	**U = 6.0**[b]
		***p* = .002**
Stationary Alert	Duration	U = 23.5
		p = .236
Eliminate	Duration	U = 22.0
		p = .20
Other Solitary	Duration	U = 22.5
		p = .20
Stereotypic	Duration	U = 26.0
		p = .37
Not Visible	Duration	**U = 7.0**[b]
		***p* = .004**

Table 2.6 (Continued)

Bleat	One-Zero	U = 28.0 *p* = .481
Honk	One-Zero	U = 32.0 *p* = .743
Visit Feeder	All-occurrence	**U = 9.5[b]** ***p* = .008**
Visit Feeder (depleted)	All-occurrence	**U = 9.5[b]** ***p* = .008**
Visit Feeder (never baited)	All-occurrence	U = 36.0 *p* = 1.0
Scent Mark – Total*	All-occurrence	**U = 17.0[a]** ***p* = .037**

*One-tailed test (*a priori* hypothesis: males > females)
[a]Males > Females
[b]Females > Males

As predicted, males locomoted more than females (see Table 2.6 and Figure 2.18) and scent marked more than females (see Table 2.6 and Figure 2.19). Both of these behaviors correspond to range expansion. I also found that males fed on bamboo for longer durations per hour than did females (see Table 2.6 and Figure 2.18). Females rested more and spent more time not visible than males (see Table 2.6 and Figure 2.18). Females also exhibited overall higher rates of visiting feeders than males, and higher rates of visiting correct (i.e., depleted feeders) (see Table 2.6 and Figure 2.19).

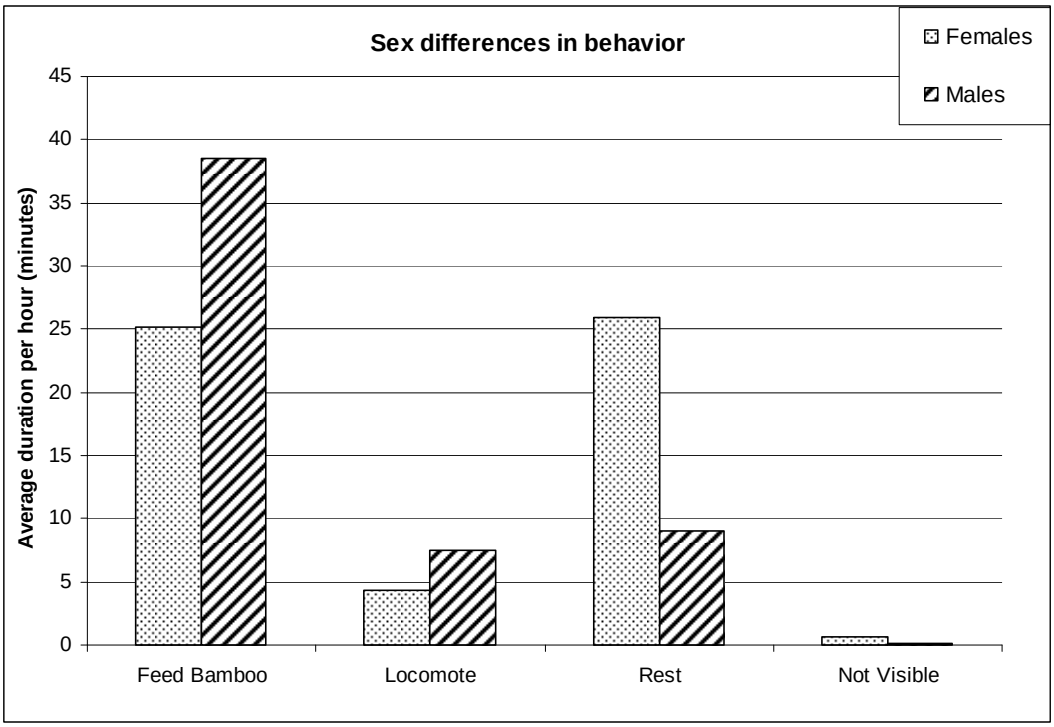


Figure 2.18. Significant sex differences in durations of behavior.

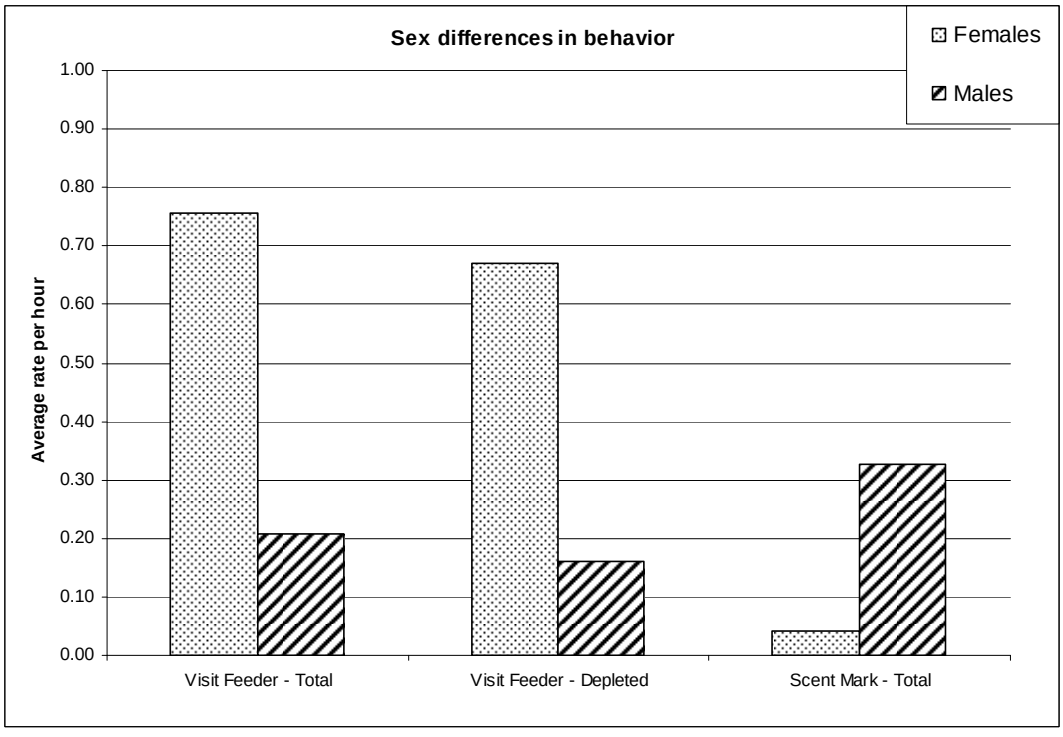


Figure 2.19. Significant sex differences in rates of behavior.

Discussion

In summary, giant pandas learned the spatial task and males outperformed females on a number of measures. From an evolutionary perspective, these results can be best explained by the range size hypothesis given that the potential influence of decreased female ability during estrus was avoided. This hypothesis proposes that sex differences in spatial ability will be observed in species with sexually dimorphic range sizes. Giant pandas have a promiscuous mating system and male ranges encompass the smaller, core areas of females; therefore, sex differences on a spatial task would be expected.

First, it is important to note that both males and females showed evidence of learning the task. Subjects tended to re-visit depleted feeders more than never baited ones in all intervals, suggesting that subjects learned the correct location information. Across intervals, all subjects showed a significant increase in the number of correct responses in the first four visits, and a decrease in re-visits to depleted locations. Finally, performance was above-chance on the probe trial. Thus, the observed differences were not because females failed to acquire the task. Furthermore, it is unlikely that sex differences in performance reflect differences in other sensory abilities. If subjects were responding to olfactory or visual cues during the test trials, performance should diminish on the probe trial. However, performance remained stable which suggests that subjects were responding based on spatial location. Thus, it is reasonable to conclude that any observed differences are based on sex differences in spatial ability.

Overall males outperformed females when considering total number of responses, reference memory errors and working memory errors. Specifically, males made fewer total responses, reference memory errors (i.e., visits to feeders that are never baited) and working memory errors to never baited feeders (i.e., re-visits to feeders that never contain food) in interval 2 and 3. The lack of a sex difference on these measures in interval 1 likely corresponds to all subjects initially learning which locations actually contained

food. In contrast, the sex difference in total working memory errors (i.e., re-visits to feeders) and working memory errors to depleted feeders (i.e., re-visits to feeders that have already been depleted in that session) was evident from the onset of testing and significant in all 3 intervals.

Previous research in rodents and primates suggests that the magnitude of the male advantage in spatial ability may be greatest for working memory tasks (Jonasson, 2005; Lacreuse et al., 2005) and testosterone is most likely responsible for this effect (Sandstrom et al., 2006). On a radial arm maze, castrated male rats committed significantly more working memory errors (Spritzer et al., 2008). Similarly, castrated male were significantly impaired on a working memory version of the Morris water maze, but equal to controls on the reference memory task. Exogenous administration of testosterone restored performance on the working memory component (Sandstrom et al., 2006). The range size hypothesis provides an evolutionary basis for understanding the greater advantage on working memory. If the sex difference relates to improved male reproductive success, as predicted by the hypothesis, there would be no obvious advantage for superior male spatial memory for unchanging characteristics of the environment (i.e., reference memory), such as the location of water sources. Instead, remembering flexible, changing information (i.e., working memory) such as the location of females or communal scent marking locations would be relevant for males. Thus, the greater advantage on working memory tasks can be interpreted from an evolutionary perspective.

In this study, sex differences in the giant pandas' performance were observed during the period of male range expansion and elevated testosterone and *outside* the female reproductive period. Thus the potentially confounding influence of decreased female spatial ability, as suggested by the fertility and parental care hypothesis (Ecuyer-Dab & Robert, 2004; Sherry & Hampson, 1997), was avoided in the current study.

Previous research has failed to distinguish between these hypotheses. Both make the same general predictions: males will outperform females and these differences will be minimal outside of periods of reproductive viability (i.e., non-breeding season or outside of sexual maturity), and the same results can be interpreted from either perspective. Therefore a test than can provide support for one hypothesis while controlling for the other is needed to advance evolutionary theory. It should be recognized that these hypotheses are not necessarily mutually exclusive and the current results do not rule out the potential influence of the fertility and parental care hypothesis. However, these findings do provide clear support for the range size hypothesis that cannot be explained by the fertility and parental care hypothesis. As predicted, in a species with a promiscuous mating system and sexually dimorphic range size, males outperformed females on a spatial task during the period corresponding to range expansion.

Behaviorally, males locomoted and scent-marked more than females. Both of these behaviors correspond to range expansion in the wild. Females did not exhibit any signs of estrus during the course of the study. This provides further support that the spatial task data were collected during the period of male range expansion and prior to female estrus. Males also spent more time feeding on bamboo than females, and females spent more time resting. Although this is speculative, it is possible that the increased food intake observed in males is also related to range expansion. More food energy might be necessary to sustain increased activity levels. Several other recorded behaviors were pertinent to the spatial task. During behavioral observations, which took place after testing, females continued re-visiting feeders at a higher rate than males, providing further support for sex differences in spatial memory.

It is possible that differences in performance result from other factors, however many of these possibilities were controlled for in the study design. For example, there might be sex differences in sensory abilities that influence performance. However I

rubbed the scent of the apple to all feeders, thus olfactory cues alone could not be used to identify baited feeders. Visual cues could also potentially aid performance. Feeders lids were attached with a spring-loaded that kept lids closed unless they were actively held open. Thus depleted feeders were not visually distinct from feeders that still contained food, and baited feeders were not visually distinct from never baited feeders. All feeders were cleaned between sessions so no other visual cues were available to guide performance. As further support that visual or olfactory cues were not controlling behavior, performance on the probe trial was above chance even when no food items were present. Another possibility is that females are more food motivated than males and continue searching for food. However, the behavioral data suggests that males actually spent more time eating than females. There may also be sex differences in stereotyped patterns of behavior that might influence performance on the task, however no differences were observed in the behavioral data. One final possibility is that males and females may have different social roles that influence performance on this task. For example, males might need to be alert or on guard for predators, and spend less time investigating a food source. However, this factor would be more likely to arise in social species. In the current study, males and females occupied similar enclosures and bamboo was always available. Thus, conditions and behavioral opportunities were identical for males and females.

This study provides compelling support for the range size hypothesis in the order Carnivora, and suggests that male giant pandas have better spatial memory than females. Further investigation of this phenomenon could include a test of males and females outside of the breeding period. We would expect the sex difference to diminish during this period. Another test would be to compare males and females that are not sexually mature. Again, we would expect the sex difference to be minimized during this period. Another interesting possibility would be to test female ability during estrus to see if this

further impairs female ability. This finding would imply that the evolutionary pressures described by the range size hypothesis and the fertility and parental care hypothesis are both influencing performance. However the estrous period is very short and food motivation may also be an issue during this period. Testing with other species may provide better insight into this question. If range expansion and female estrus do overlap, both sexes could be tracked over a long period of time that includes multiple range expansions and multiple estrous periods. This would require a modified task that was not characterized by a period of acquisition followed by a steady state of performance. One possibility would be to bait certain locations in a morning session, and re-bait those feeders in the afternoon. The next day a different set of feeders would be baited and then repeated in the afternoon. Although this would not allow for an exploration of reference memory, comparisons of working memory could be made. Specifically, performance could be compared within sexes with the expectation that performance would decrease during the relevant periods (i.e., range expansion or estrus). A final possibility would be to measure estrogen and androgen levels, and then determine whether these hormonal values correlate with performance on a spatial task. All of these suggestions could be used to further our understanding of the evolution of sex differences in spatial ability in giant pandas.

CHAPTER 3

SEX DIFFERENCES IN OTTER SPATIAL MEMORY

The range size hypothesis predicts that sex differences should only be observed in species in which the mating system has selected for a differential mating system. For some mating systems, such as monogamy, males and females occupy equal range sizes. In these species, no sex difference would be predicted because there should be no selective advantage or reproductive benefit of differential spatial ability. Thus, a thorough evaluation of the range size hypothesis would also require a test of a monogamous species, in which no sex differences would be expected. However, as few as 7% of species in the Order Carnivora adhere to a monogamous mating system (Dalerum, 2007). Although there are no monogamous bear species for comparison, there are some monogamous species in the Order Carnivora that can be used to make useful comparisons to giant pandas and further test the range size hypothesis.

The Asian small-clawed otter is one of the closest relatives of the giant panda that adheres to a monogamous mating system. Fifty-two million years ago, the carnivore ancestor split into caniformia ("dog-like branch) and feliformia ("cat-like branch"). Within caniformia, there was a split forty-three million years ago between cyonedia (wolves, dogs, foxes) and artoidea ("bear-like carnivores") (Arnason, Gullberg, Janke, & Kullberg, 2007). The artoidea branch consists of ursids, mustelids, and pinnepids. There are no monogamous species in the ursid family, but there are several in the mustelid family (Johnson et al., 2000), including Asian small-clawed otters (Aonyx cinereus).

Asian small-clawed otters are the smallest otter species. They live in a wide range of habitats throughout southeast Asia (Sivasothi & Burhanuddin, 1994). Males and females form a monogamous bond and mate for life. Females undergo multiple, brief, aseasonal estrous periods each year (Bateman et al., 2009). Gestation is approximately 60 days and the average litter size is 4.4. Otters are sexually mature by one year of age (Lariviere, 2003). Asian small-clawed otters live in family groups of approximately 10-12 individuals and older siblings help raise younger ones. Males and females jointly rear offspring and share a home range (Lariviere, 2003; Sivasothi & Burhanuddin, 1994).

Very little empirical research has focused on otters (Sivasothi & Burhanuddin, 1994). In particular, there has been almost no research on otter cognitive abilities, although anecdotal reports suggest that this is a highly intelligent species. For example, Asian small-clawed otters have been reported to gather clams and lay them in the sun until the heat causes them to open even though the otters are capable of cracking them open immediately (Sivasothi & Burhanuddin, 1994). In southeast Asia, fisherman train this species of otter to swim and drive fish into nets to assist with fishing efforts. Other species of otter have been reported to use rocks as tools to break open shells. Asian small-clawed otters have partially webbed paws with small, rudimentary claws (Sivasothi & Burhanuddin, 1994). They are very manually dexterous and use their paws to locate and obtain food, even when hidden under rocks. Otter spatial memory has never been tested, but a task that requires subjects to reach into a feeder to obtain food would be suitable for testing with Asian small-clawed otters. I used a simulated foraging task to test spatial memory. Based on the range size hypothesis, I predicted that males and females would perform similarly on a simulated foraging task.

Method

Subjects

I tested 9 Asian small-clawed otters (4 male, 5 female) housed at Zoo Atlanta from May-June 2010 (See Table 3.1). One female did not consistently participate in the task, and her data were dropped from the analysis.

Table 3.1. Otter subjects, sex, and date of birth

Name	Studbook Number	Sex	Date of Birth
Harry	1973	F	3/6/2005
Merrill	1874	F	3/6/2005
Modine	1895	F	9/29/2005
Nava	1649	F	10/29/2000
Tinsley[a]	1875	F	3/6/2005
Brownie	1894	M	9/29/2005
Bugsy	1892	M	9/29/2005
Lil' T	1896	M	9/29/2005
Moe	528	M	9/13/1993

[a]Subject not included in analysis

Apparatus

A feeder consisted of an "elbow PVC pipe connector" attached to a corner bracket so that each feeder could be screwed into the concrete flooring substrate. Feeders were designed so that depleted feeders were not visually apparent or distinct from other feeders (Figure 3.1).

Figure 3.1. Otter feeder

Eight feeders were arranged in a circular pattern (see Figure 3.2). Each feeder was rubbed with the scent of the food item (fish) before each session so that the baited feeders could not be identified by olfactory cues. Feeders were cleaned between sessions.

Figure 3.2. Feeders were placed equidistantly in a circular pattern.

Figure 3.3 Otters retrieved fish by reaching into the feeder, grasping the food item, and pulling it out.

Procedure

For each session, the same four feeders were baited with the fish, and the other four were left empty. Subjects were allowed to freely explore the feeders. Any contact with a feeder was recorded as a visit, and the pattern of visitation was recorded for each session. The day after the test sessions, a "probe trial" was conducted in which all feeders were rubbed with the scent of the food item, but none were baited with food. The probe trial further tested the possibility that subjects were using extraneous cues (e.g., scent) rather than spatial cues to find food in the previous task. Only four visits were needed to recover all food items, so the proportion of the first four responses to correct locations was determined. If this performance fell below chance when food was absent, subjects may have been relying on cues from the food itself. However, if performance remained above chance on the "probe trial," then it was concluded that spatial location was the relevant information used to solve the task.

Data Analysis

A number of measures were recorded (see Table 3.2). For each of these measures, test trials were summarized into 3-session intervals, resulting in seven intervals.

Did subjects learn the task?

Performance on the probe trial was compared to chance performance using a one-sample t-test ($\alpha = .05$). No food was present during the probe trial. If subjects were responding based on spatial location, and not on visual or olfactory cues, performance on the probe trial should be above chance.

I also compared whether subjects revisited correct (i.e., depleted) or incorrect (i.e., never baited) locations during a session. The number of re-visits to depleted feeders was compared to the number of re-visits to feeders that were never baited using a

nonparametric paired samples test, the Wilcoxon signed ranks test ($\alpha = .05$). If subjects were learning the spatial location of food items, it was expected that subjects would return to depleted feeders more often than never baited feeders.

I also tested whether performance on all measures improved across sessions using a nonparametric repeated measures test, Friedman's test ($\alpha = .05$).

Sex differences?

For each interval, the performance of males and females was compared using a two-tailed Mann-Whitney U test for each measure ($\alpha = .05$). To analyze the behavioral data, a Chi-square goodness-of-fit test was used to compare the distribution of various behaviors across males and females. I chose this more conservative test because of the small sample size and to control for any error in identifying individual otters during behavioral data collection. It was sometimes impossible to distinguish between individual otters when they were on exhibit, but it was possible to reliably and consistently differentiate between males and females because of size and morphological differences. Therefore the Chi-square test ($\alpha = .05$) was used to compare the distribution of behaviors across males and females to test for sex differences in locomotion. This analysis was conducted on all behavioral measures.

Table 3.2. Spatial task measures and definitions

Measure	Definition
# Correct in First Four Visits	Number of visits to baited location in first four visits. Only four visits are needed to locate all food items.
Reference Memory Error	Visit to a location that is never baited with food.
Working Memory Error	Re-visit to a location that has already been visited in that session.
Working Memory Error – Depleted Feeder	Re-visit to a correct location (baited with food) that has already been visited in that session.
Working Memory Error – Never Baited Feeder	Re-visit to an incorrect location (never baited with food) that has already been visited in that session.
Total Number of Visits	Number of visits within a session (within 20 minutes of the first visit)
First Four Visits	Number Correct in the first four visits

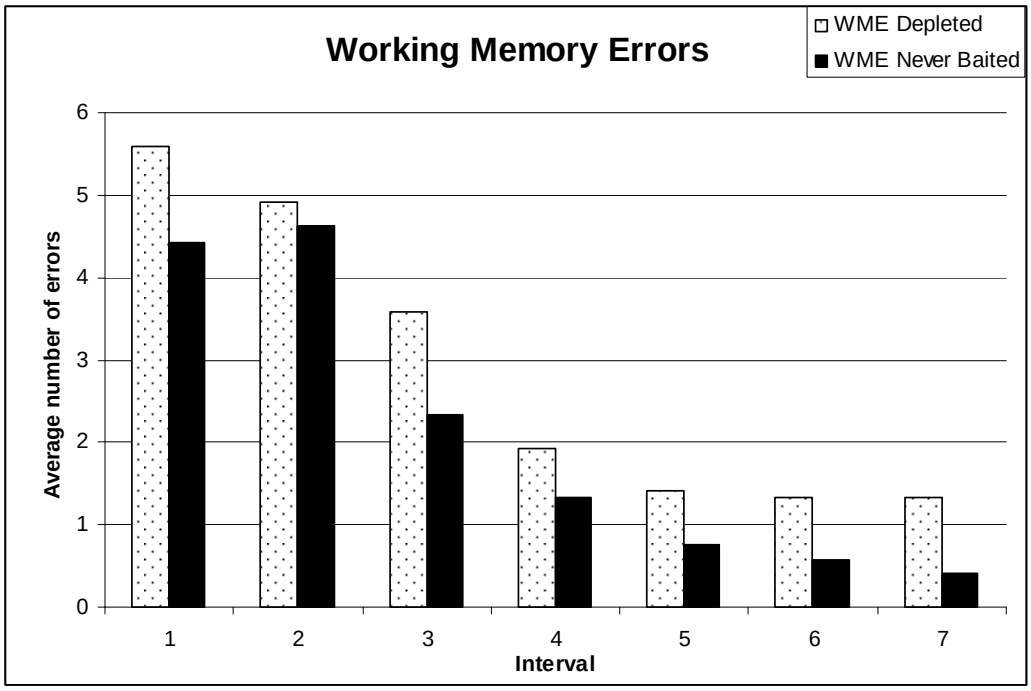
Results

Did subjects learn the task?

On the probe trial, otters ($t = 2.39$, $df = 7$, $p < 0.05$) were significantly more likely than chance to visit to correct locations (i.e., baited on previous trials) in the first four visits, even though no food was present. Subjects re-visited depleted feeders significantly more often than never baited feeders in 5 out of 7 intervals (see Table 3.3 and Figure 3.4).

Table 3.3. Working memory errors to depleted or never baited feeders.

Interval	Wilcoxon Signed Ranks test
1	**Z = -2.375** **p = .018**
2	Z = -.426 p = .670
3	**Z = -2.55** **p = .011**
4	Z = -1.479 p = .139
5	**Z = -2.263** **p = .024**
6	**Z = -2.375** **p = .018**
7	**Z = -2.539** **p = .011**



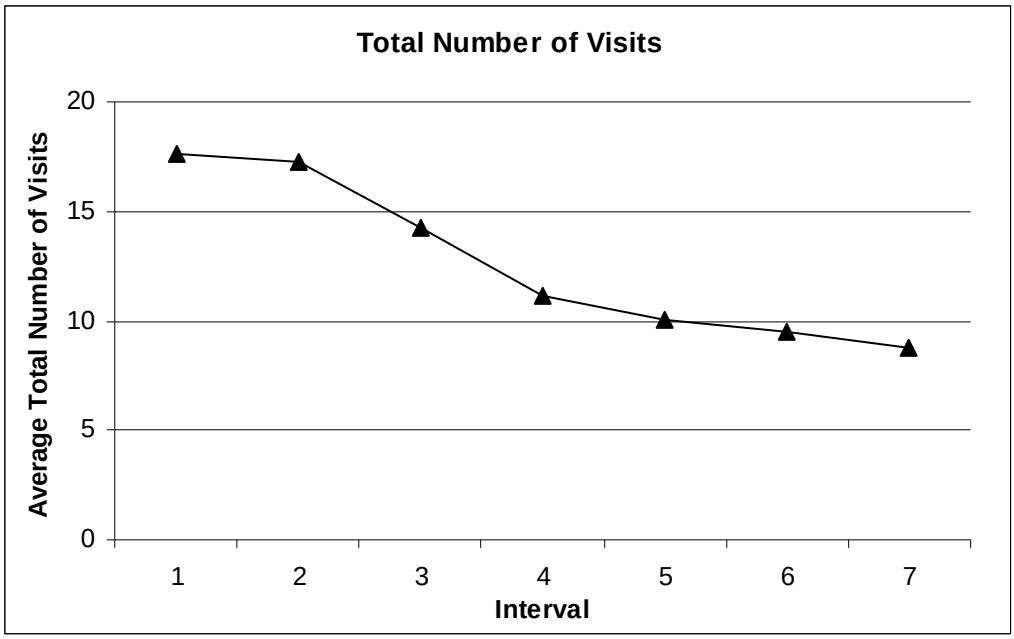
*significant difference in intervals 1, 3, 5, 6, 7

Figure 3.4. Difference in working memory errors to depleted or never baited feeders

There was significant improvement across sessions for all measures (see Table 3.4. and Figures 3.5 – 3.10).

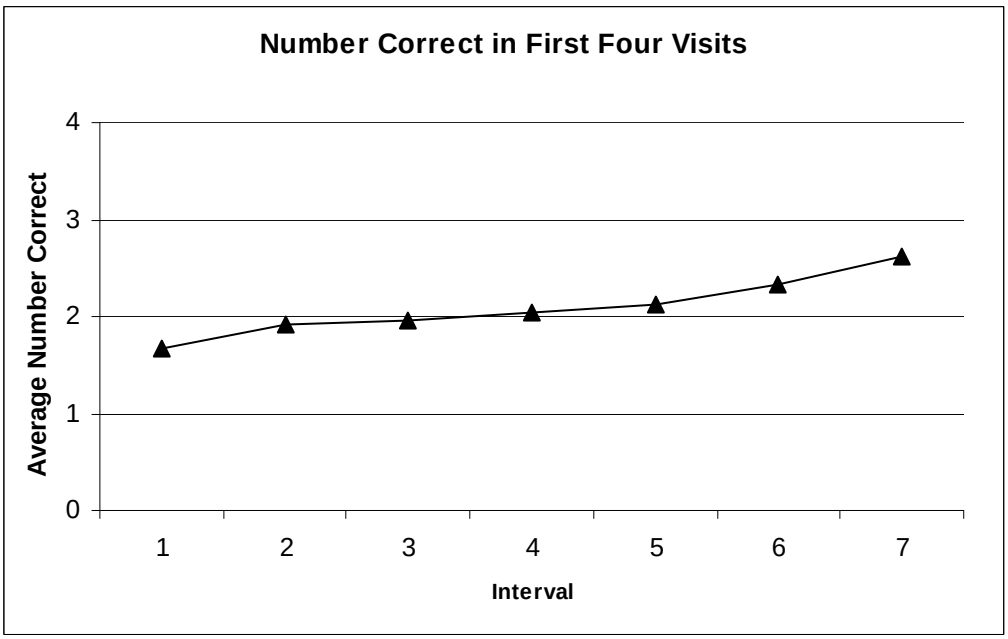
Table 3.4. Change across intervals

Measure	Friedman's test
Total Number of Visits	$\boldsymbol{X^2}$ **= 39.291** $\boldsymbol{p}$ **= .000**
# Correct in First Four Visits	$\boldsymbol{X^2}$ **= 23.506** $\boldsymbol{p}$ **= .001**
Reference Memory Error	$\boldsymbol{X^2}$ **= 18.946** $\boldsymbol{p}$ **= .004**
Working Memory Error	$\boldsymbol{X^2}$ **= 38.023** $\boldsymbol{p}$ **= .000**
Working Memory Error – Depleted Feeder	$\boldsymbol{X^2}$ **= 39.822** $\boldsymbol{p}$ **= .000**
Working Memory Error – Never Baited Feeder	$\boldsymbol{X^2}$ **= 36.984** $\boldsymbol{p}$ **= .000**



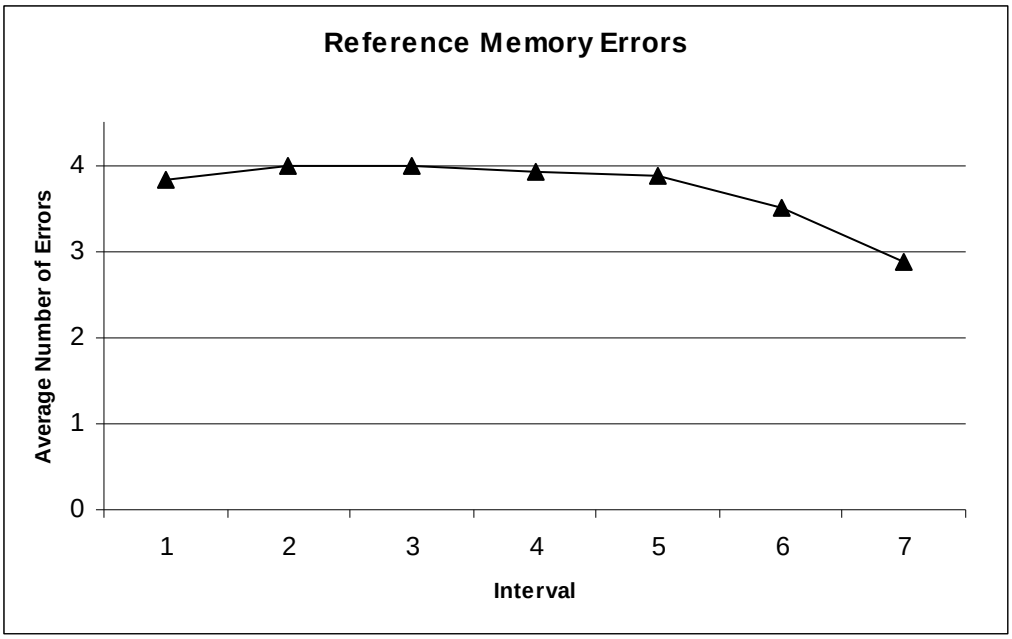
*significant decrease

Figure 3.5. Total number of visits across intervals.



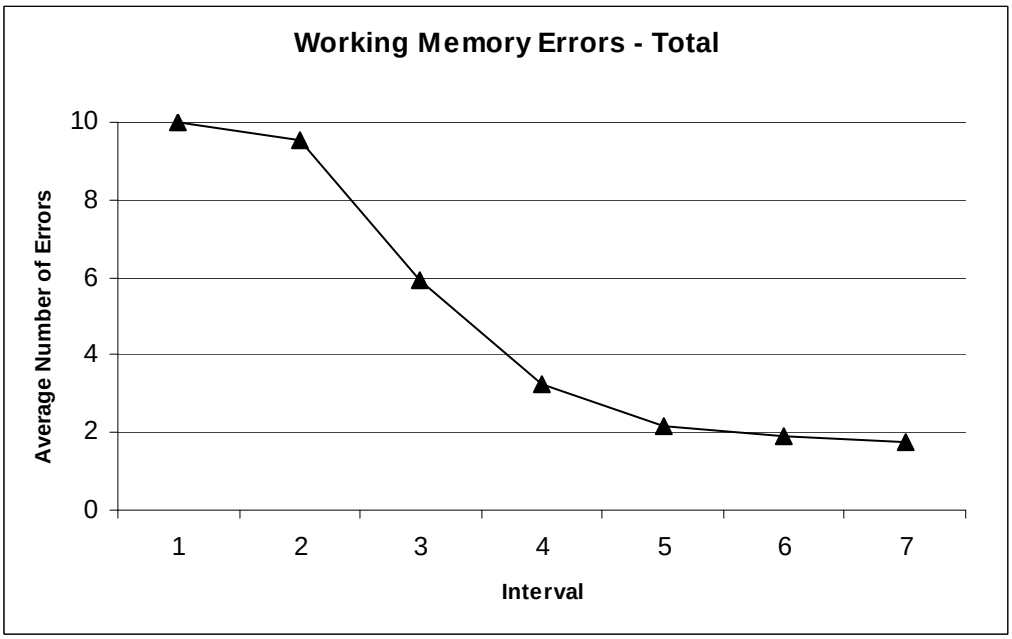
*significant increase

Figure 3.6. Number correct in first four visits across intervals



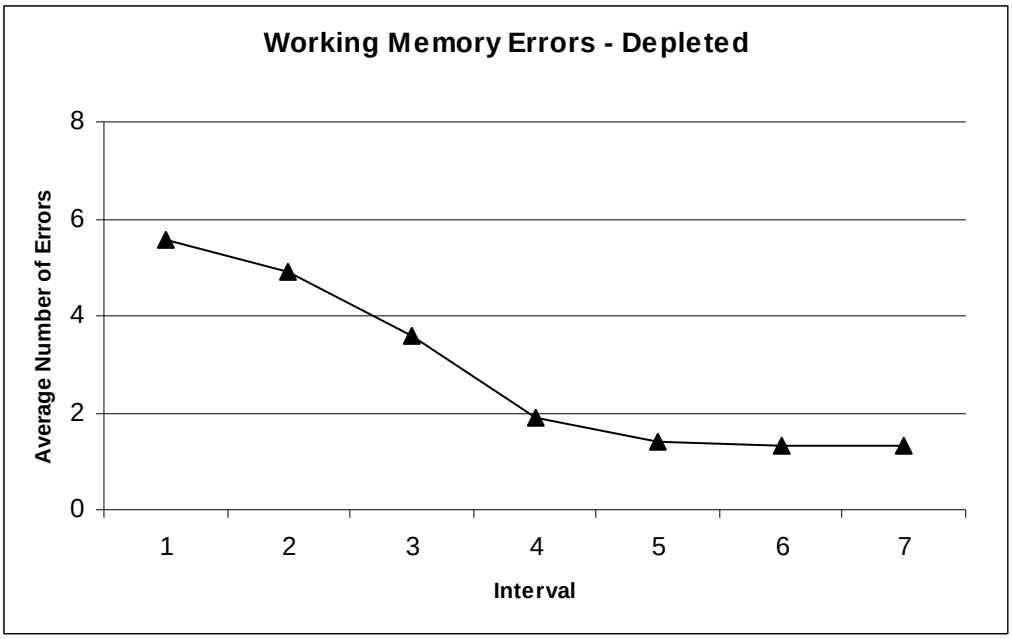
*significant decrease

Figure 3.7. Reference memory errors across intervals.



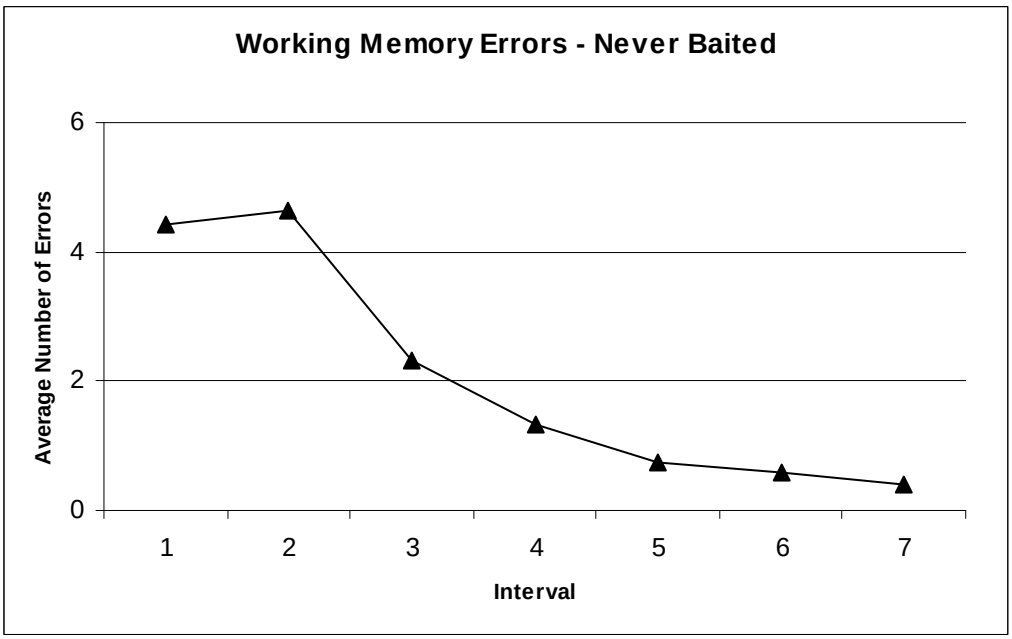
*significant decrease

Figure 3.8. Working memory errors (total) across intervals.



*significant decrease

Figure 3.9. Working memory errors (depleted) across intervals



*significant decrease

Figure 3.10. Working memory errors (never baited) across intervals.

Sex differences?

There were no significant sex differences in otter performance on the spatial memory task ($p > .05$ in all intervals; Table 3.5; Figures 3.11 – 3.16).

Table 3.5. Mann-Whitney U statistic and p-value (two-tailed) for all measures

Measure	Statistic	Interval						
		1	2	3	4	5	6	7
Total # of Visits	U	6.0	4.0	8.0	5.0	6.5	3.0	3.0
	p-value	.686	.343	1.0	.486	.686	.20	.20
# Correct in First Four Visits	U	7.0	4.0	6.0	6.0	6.0	6.5	6.0
	p-value	.886	.343	.686	.686	.686	.686	.686
Reference Memory Error	U	8.0	8.0	8.0	8.0	7.5	6.0	4.5
	p-value	1.0	1.0	1.0	1.0	.886	.686	.343
Working Memory Error	U	6.0	6.0	8.0	5.0	7.0	3.5	6.0
	p-value	.686	.686	1.0	.486	.886	.200	.686
Working Memory Error – Depleted	U	6.0	2.0	6.0	5.5	7.0	5.0	7.5
	p-value	.686	.114	.686	.486	.886	.486	.886
Working Memory Error – Never Baited	U	5.5	6.0	7.5	4.0	5.0	3.0	6.0
	p-value	.486	.686	.886	.343	.486	.200	.486

Although the otter sample size is small, statistical analysis and visual inspection of the data reveal absolutely no trend towards sex differences in performance on the spatial task.

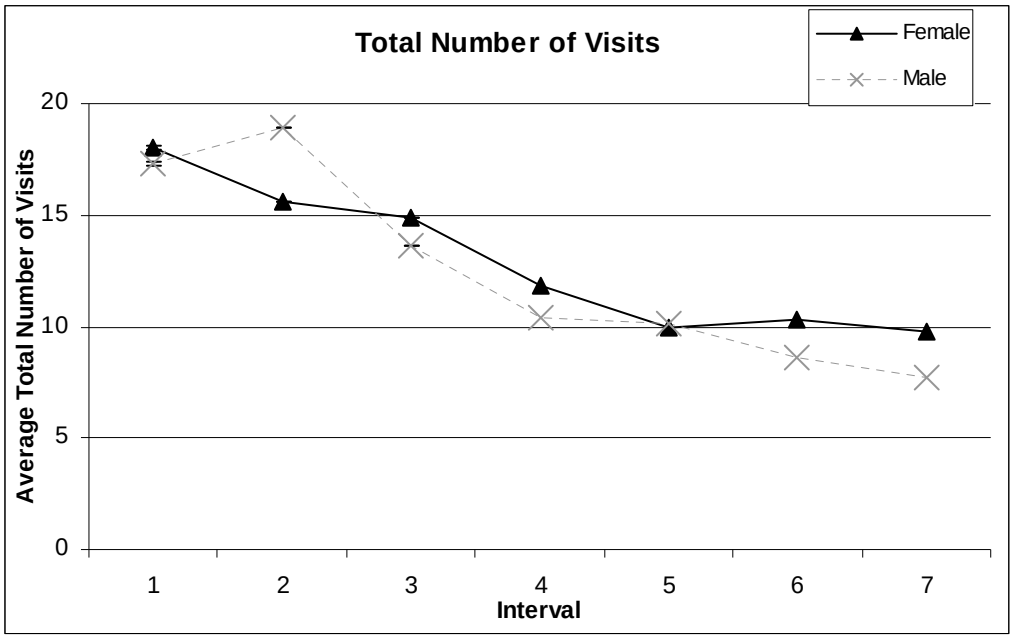


Figure 3.11. Sex differences in the total number of visits

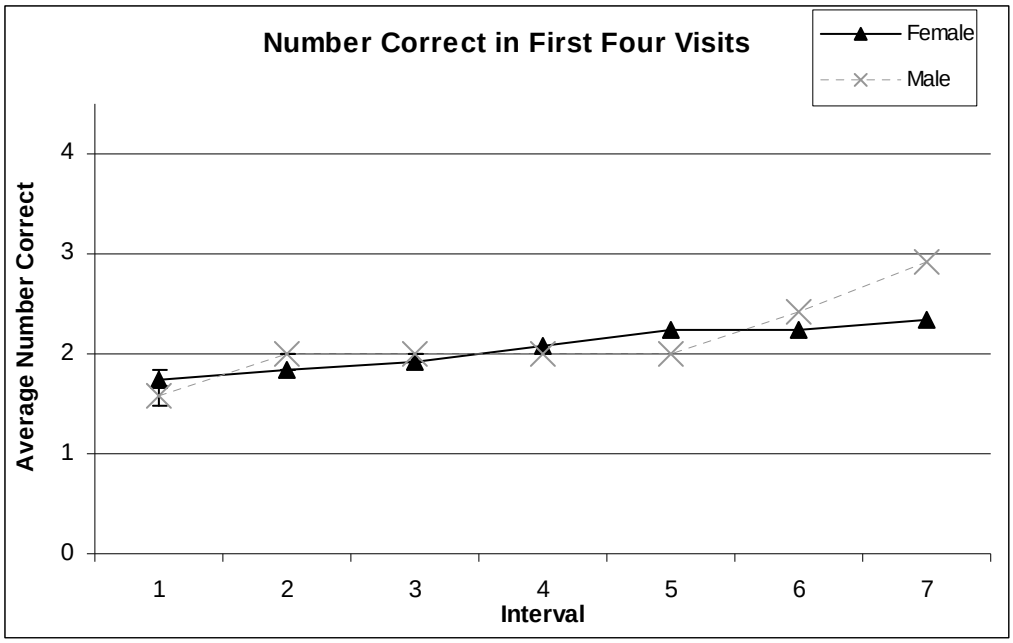


Figure 3.12. Sex differences in the number correct in the first four visits

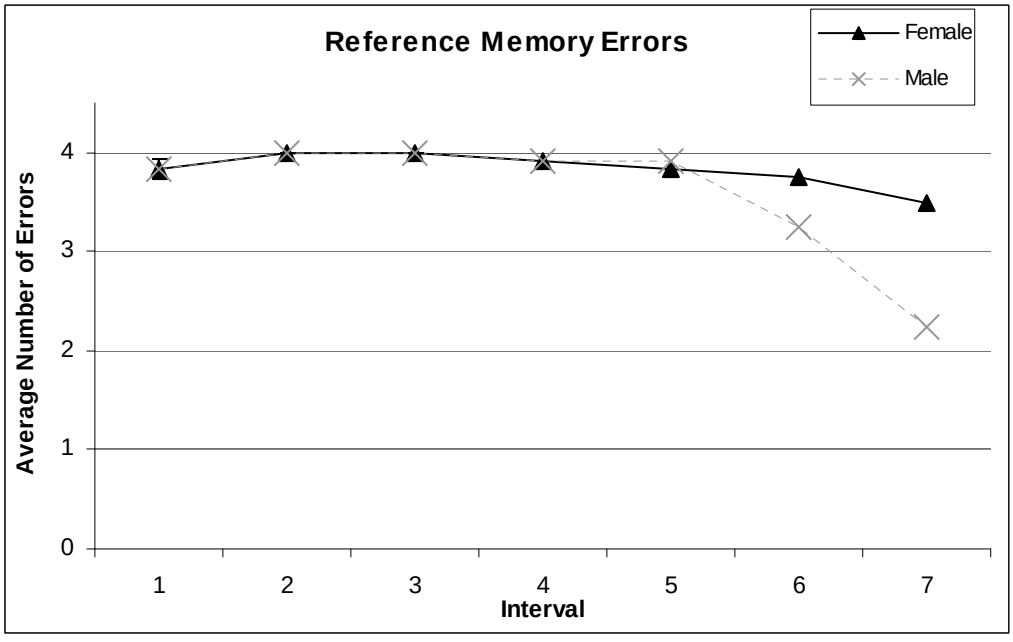


Figure 3.13. Sex differences in reference memory errors

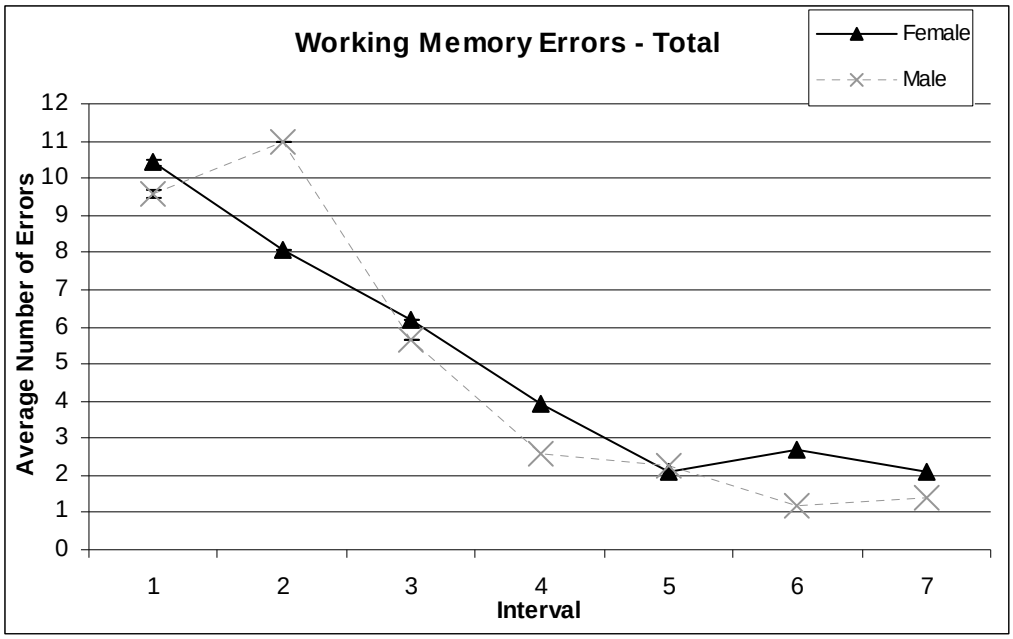


Figure 3.14. Sex differences in working memory errors (total)

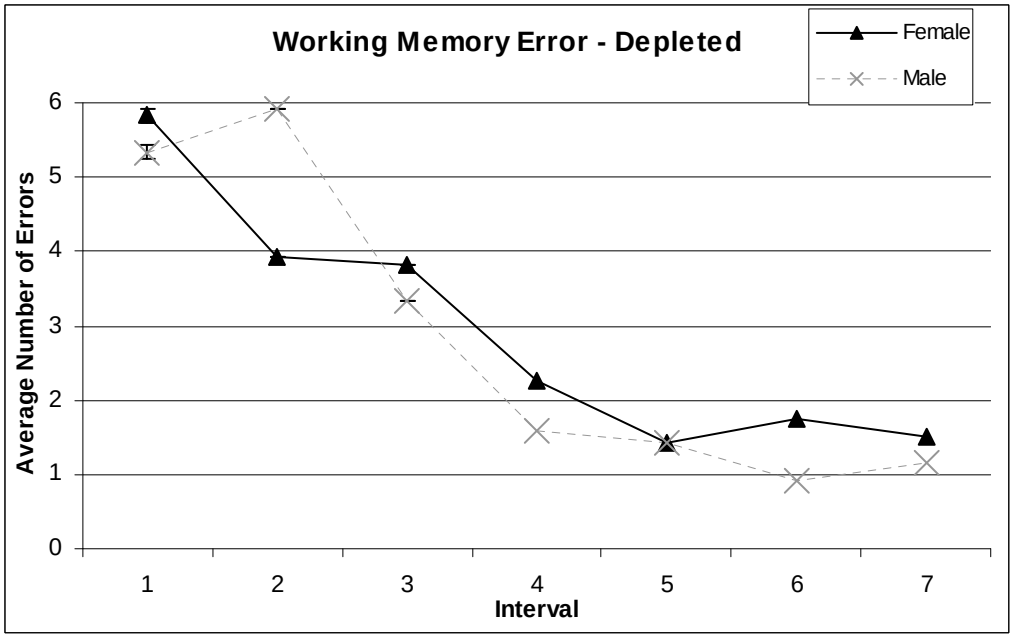


Figure 3.15. Sex differences in working memory errors (depleted)

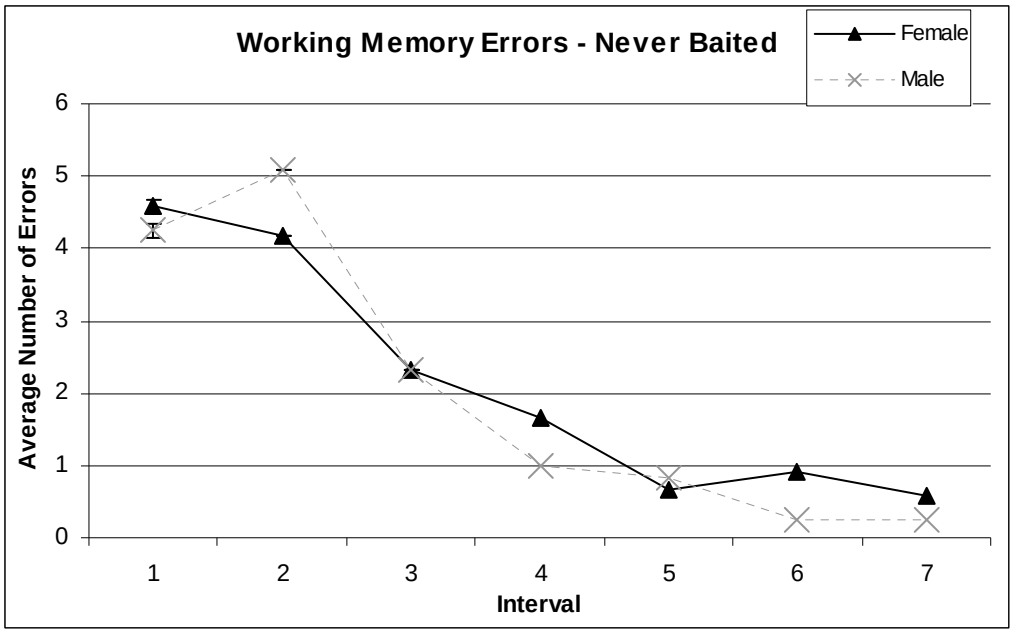


Figure 3.16. Sex differences in working memory errors (never baited)

There were no sex differences in the distribution of behaviors with the exception of "other solitary" (see Table 3.6).

Table 3.6. Sex differences in behavioral measures.

Behavior	Chi-Square
Locomote	$X^2 = .10$ $p > .05$
Maintanence	$X^2 = 0.01$ $p > .05$
Rest	$X^2 = 0.55$ $p > .05$
Feed	$X^2 = 0.08$ $p > .05$
Forage	$X^2 = 1.94$ $p > .05$
Eliminate	$X^2 = 0.69$ $p > .05$
Other Solitary	$\mathbf{X^2 = 6.22}$ $\mathbf{p > .05}$
Swim	$X^2 = 1.86$ $p > .05$
Stationary Alert	$X^2 = 0.02$ $p > .05$
Nest	$X^2 = 0.42$ $p > .05$
Door directed	$X^2 = 0.00$ $p > .05$
Other Social	$X^2 = 0.01$ $p < .05$
Social Play	$X^2 = 0.48$ $p > .05$
Not Visible	$X^2 = 0.05$ $p > .05$
Locomote + Swim	$X^2 = 1.96$ $p > .05$

Discussion

As predicted by the range size hypothesis, there were no sex differences in Asian small-clawed otter performance on the spatial task. This finding is consistent with previous research in monogamous species (Gaulin & Fitzgerald, 1986, 1989; Langley, 1994). All subjects showed evidence of learning the task. Across intervals, there was a significant increase in the number correct in the first four responses. There was a significant decrease in the total number of responses, reference memory errors, total working memory errors, working memory errors to both depleted and never baited locations. Subjects re-visited depleted feeders more than never baited ones in 5 out of 7 intervals, further suggesting that subjects learned the correct location information. Finally, performance was above-chance on the probe trial. If subjects were responding to olfactory or visual cues during the test trials, performance should diminish on the probe trial. However, performance remained stable which suggests that subjects were responding based on spatial location. Thus, otters successfully learned the correct spatial locations in a simulated foraging task. Therefore the complete lack of sex differences on the task can be considered an accurate reflection of ability. Although it is possible that a sex difference might emerge with continued testing, males typically outperform females during the acquisition of spatial tasks (McCarthy & Konkle, 2005). In giant pandas, the difference in working memory errors was evident from the onset of testing. Over twice as many test sessions were conducted with otters, so it can be concluded that no sex differences were observed during the same phase of testing in which giant panda differences were observed.

These data provide support for a critical component of the range size hypothesis: the predicted *lack* of sex differences in a monogamous species. As with many other fields of inquiry, the focus of the literature is all too often based around significant differences, even when a lack of difference can be equally as meaningful. This research highlights

the importance of testing species with equivalent range sizes with the prediction of equal spatial ability. By incorporating all relevant information, not just statistically significant data, we can advance evolutionary theory. Within the order Carnivora, several other species, such as meerkats (*Suricata suricatta*) and gray wolves (*Capis lupus*), have mating systems that have selected for equal range sizes and provide interesting opportunities for future research. If the range size hypothesis is correct, we would expect males and females of these species to perform equally well. Another interesting possibility for future research would be to make comparisons between various otter species. Several of the 13 otter species exhibit intra-sexual territory patterns where males inhabit larger ranges that encompass female ranges. For example, male Cape clawless otter (*Aonyx capensis*) ranges encompass the ranges of females (Somers & Nel, 2004). The range size would predict sex differences on a spatial task for this closely related otter species.

Gaining knowledge about otters is important because they are often considered to be an "indicator" species that reflect the health of an environment. They are sensitive to water quality and their presence and behavior reflect the effect of pollution on aquatic habitats. As a result of human activity, many otter species are vulnerable or threatened. Otters presently live in a variety of habitats (Sivasothi & Burhanuddin, 1994) and inhabit all continents except for Australia and Antarctica (Kruuk, 1994). They show substantial variation in behavior and ecology within species, populations and even at the individual level (Kruuk, 1995). By studying otters, we can learn a great deal about ecology, resources, and various behaviors such as playing, hunting, scent-marking and tool use (Kruuk, 1995). In the wild, otters are often hard to study because they are nocturnal, secretive and live in hard to reach habitats (Kruuk, 1995). Testing in a captive environment provides a useful opportunity to learn more about this species. By learning more about otter behavior and cognition in captive environments, we can improve

conservation efforts and educate people about this species, which will hopefully motivate changes in human behavior that will ensure the survival of this species.

CHAPTER 4

OVERALL DISCUSSION

This study provides the first support for the range size hypothesis in the order Carnivora. Male giant pandas outperformed females on a spatial foraging task. The range size hypothesis also predicts that no sex differences will be observed in a monogamous species with equal range sizes. Despite the close phylogenetic relationship to the giant panda within the order Carnivora, no sex differences were observed in Asian small-clawed otter performance. These findings support the hypothesis that different mating systems and ranging patterns are predictive of sex differences in spatial ability. In addition to advancing evolutionary theory regarding sex differences in cognition, this work illustrates the usefulness and importance of integrating across proximate and ultimate levels of explanation, testing in captivity, and how zoos provide a unique forum for advancing cognition research.

Evolution of Sex Differences in Spatial Ability

In many species, males are better able to navigate the environment and remember spatial information than females, and the current data contribute to this body of knowledge. The most prominent explanations regarding sex differences in spatial ability explain the dimorphism in terms of sexual selection. Sexual selection relates to variations in reproductive success that drive the evolution of certain characteristics. Two main processes of sexual selection are intrasexual competition and intersexual choice. The sex that invests less in offspring can benefit from mating with multiple partners. The sex that invests more does not benefit from additional partners, and is more likely to be choosy in

accepting a mating partner. This sex becomes a limiting resource. In any species with internal gestation and obligate postnatal care, the female typically invests more in offspring (Geary, 1998). Thus, females are generally the limiting resource, and males must compete for access. Males may physically compete as individuals or in coalitions, or they may compete through secondary sex characteristics (female choice may be biased towards males with enhanced secondary sex characteristics), or through territory establishment and maintenance.

The range size hypothesis (Gaulin & Fitzgerald, 1986. 1989; Gray & Buffery, 1971) suggests that in promiscuous or polygynous mating systems, males can benefit from expanding their range during breeding season. Rather than directly guarding females, males expand their range and monitor multiple females during the breeding season. Males that are best able to remember the location of fertile females will be more reproductively successful. This form of scramble polygyny is most successful when females are dispersed and fertile periods are asynchronous (Jacobs, 1996). As previously discussed, the key predictions are that sex differences will be evident in species in which the sexes exhibit differential range sizes as a result of mating system (promiscuous, polygynous), but species in which the sexes exhibit identical home ranges (monogamous) will not show sex differences. The current research contributes to the existing body of literature supporting for the range size hypothesis. There are two compelling lines of support for the idea that sexual selection shaped differential spatial ability: across-species comparisons and within-species variability (e.g., the sex difference only emerges during periods of reproductive viability).

Several studies have examined differential spatial ability across related species. Polygynous meadow voles show a sex difference in spatial ability, but closely related monogamous pine and prairie voles do not show sex differences (Gaulin & Fitzgerald, 1986, 1989). The primary difference between these species is the mating system. For monogamous species, there would not be a reproductive benefit of attempting to mate

with multiple females, thus intramale competition in the form of range expansion would not be a relevant selection pressure. Accordingly, monogamous voles do not show sex differences, but polygynous voles do. Another comparison has been made between deer mice & desert kangaroo rats. Polygynous deer mice exhibit sex differences, but monogamous desert kangaroo rats do not show sex differences (Galea et al. 1996; Langley, 1994). Finally, anthropological and comparative studies suggest that humans have a polygynous mating system and human males outperform females in spatial navigation tasks, providing more support for the sexual selection of spatial ability. The current study found across-species support within the order Carnivora that are consistent with previous research.

Another line of support that differential spatial ability has been shaped by sexual selection relates to within-species variation. The sex difference is most apparent during periods of reproductive viability. In other words, this difference emerges when there is potential for sexual reproduction, suggesting a direct link to sexual selection. Galea et al. (1996) demonstrate that sexually dimorphic learning is not present in pre-pubescent voles or deer mice. The sex difference does not emerge until the rodents are reproductively viable. Some reports in human and non-human primates suggest that the sex difference disappears in older age when subjects are no longer reproductively viable (Lacruese et al. 1999). It has also been reported that the sex difference is reduced substantially outside of the breeding season. Polygynous meadow voles outperformed females during breeding season, but the difference was non-significant during non-breeding season (Gaulin & Fitzgerald, 1989). I specifically tested only sexually mature adults during the period of male range expansion to maximize the likelihood of identifying a sex difference if present. During breeding season, males expand their ranges significantly in many polygynous species. This range expansion enables an individual to monitor additional female ranges and improve reproductive success. Although additional testing during non-breeding periods would be necessary to provide thorough within-species support, the

current findings are consistent with previous research given that the sex difference was observed in sexually mature subjects during a reproductively relevant period of the year.

Integrating Across Proximate and Ultimate Explanations of Behavior

Behavior, or differences in behavior, can be explained on at least four levels: mechanism, ontogeny, function, and evolution (Tinbergen, 1963). Of the many inter-disciplinary fields of research, sex differences in spatial ability may have received the most attention at each of these levels. However, there has been little overlap or integrating across levels despite the knowledge that these levels are not mutually exclusive.

Proximate Factors

Mechanistic and ontogenetic factors have a proximate influence on behavior. A great deal of research has focused on the interaction between hormones, the brain, and behavior in understanding sex differences in spatial ability. Hormones may have a long-lasting, permanent effect (organizational) or a temporary reversible effect (activational) on the organization of the brain and behavior (Eckel et al., 2008). There is a great deal of data on hormones and sex differences in cognition, but a clear understanding of the relationship is still lacking. Recent speculation suggests that both estrogens and androgens have an inverted-U shape relationship with cognitive performance. There is an optimal level, above or below which performance will be impaired. This finding may apply to organizational and activational levels of hormones, suggesting that there is an optimal level for each of these effects.

Organizational effects occur during critical periods (pre and peri-natally) and cannot be reversed. Williams, Barnett and Meck (1990) castrated male rat pups immediately after birth. Females were administered high doses of estradiol benzoate (a synthetic derivative of testosterone). The altered females (given a male-like hormonal experience) performed as well as control males on a spatial task, and the altered males

(given a female-like hormonal experience) performed similarly to control females. This suggests that hormones, rather than genetic sex, are the best predictor of later spatial performance (Williams, Barnett, & Meck, 1990; Williams & Meck, 1991).

Hormones may also have activational effects which occur later in life, are temporary, and reverse once the hormone is removed. Several studies report that subjects castrated later in life are impaired on maze tasks as compared to controls. Castration later in life prevents the release of circulating testosterone. Research has found that castrated rats are impaired on working memory aspects of spatial tasks, but that performance can be restored to the level of control subjects by exogenously administering testosterone (Spritzer et al. 2008; Sandstrom et al. 2006). These studies suggest that the activational effects of testosterone may be particularly relevant to performance on spatial working memory tasks. There is also some support that estrogens influence performance on cognitive tasks (Hampson, 2008). Naturally high levels of estrogen impair performance on working and reference memory tasks, and low levels may facilitate working memory (Galea et al. 2008). Accordingly, when testing for sex differences, it is important to consider and control for reproductive stage of females.

Hormones may influence behavior through physiological interactions with the brain. Hormones can have genomic or non-genomic effects on neurons. Receptors for androgens and estrogens are found throughout the brain, with a high density in the medial temporal lobe (MTL). The hippocampus is a structure within the MTL that plays a critical role in learning and memory. Castration results in a 50% decline in the dendritic spine density of the CA1 of the hippocampus. This effect can be reversed by administering testosterone (Sandstrom et al., 2006). Estrogens can enhance long-term potentiation, dendritic spine density, and neurogenesis, all of which are important for learning and memory (Galea et al. 2008). Thus, hormones have a direct effect on the neuromolecular processes involved in learning.

A number of studies illuminate the influence of hormones on a particular aspect of cognitive processing: cue use. This finding suggests a link between hormones and strategy or cue relevance (Hampson, 2008). Williams and Meck (1990) found that female rats (and male rats castrated at birth) relied on geometric and landmark cues when navigating a water maze. Male rats (and females treated with estradiol) relied only on the geometric layout of the room. Thus, biological sex, as influenced by prenatal hormones, predisposes males and females to differing strategy use. In humans, males tend to rely on the Euclidean geometry of the environment, and females navigate using landmarks (Saucier et al., 2002).

Although the current research did not directly investigate hormones, some inferences regarding this issue can be made based on the design of the study. Previous studies have used wild-captured subjects (e.g., Gaulin & Fitzgerald, 1986 Gaulin & Fitzgerald, 1989), thus it is unclear whether in the sex difference arises because of the actual experience of inhabiting differential ranges, or whether this difference would emerge as a result of hormonal variation irrespective of an animal's experiential history. To some extent, the findings emphasized the importance of hormones as a critical proximate cause of sex differences because the study design controlled for experiential factors, such as space use. Increased use of space during range expansion has been implicated as a potential contributing ontogenetic mechanism to the sex differences observed in spatial cognition. In the current study all subjects were raised in captivity, and males and females occupied enclosures with equivalent spatial parameters. Despite male giant pandas having never actually inhabited a larger range than females, sex differences were still observed which emphasizes the importance of biological factors in the ontogeny of sex differences in spatial ability.

However, these results can also be used to refine our understanding of the potential role of differential space use in the development of sex differences. Although the results of the current study suggest that biological factors are the primary determinant,

some aspects of differential space use may be influential as well. The concept of "space use" can be broken into at least two components: physical movement through space and processing different areas of space. In the wild, it would be impossible to determine which of these components, either increased movement through space or increased processing of different of spatial information, was critical in the development of sex differences. During true range expansion, a male would simultaneously be moving through space more *and* be exposed to varying spatial information as they explored novel space. In the current study, male giant pandas locomoted more than females, suggesting that they move through space more, even though the same amount of space is provided. Hence this study essentially controlled for the actual space available, and only the movement through space differed between males and females. This finding suggests that if "space use" plays a role in the development of sex differences, it is likely movement through space, and not exposure to varying spatial configurations, is the primary contributing variable.

There are also neural structures that might be particularly relevant for understanding sex differences in cognition. In particular, the hippocampus is a region of the brain that has been implicated in spatial memory and processing spatial information. In species that show differential spatial ability, this brain region is affected. In polygynous voles, the male hippocampus was 3.3 mm^3 larger than the female hippocampus during the breeding season. In monogamous voles, the male hippocampus was .2mm^3 larger than the female hippocampus, which can be explained by the larger body size of males. Although morphological size is a crude measure of brain function (Sherry, 2006), it likely relates to more relevant measures such as connectivity, number of neurons, and rates of neurogenesis and long term potentiation. Thus, variation in brain size provides a potential neurological mechanism for differential spatial ability. Although neurological research with giant pandas is currently not possible, it is likely that the hippocampus is involved in spatial processing and future work might be aimed at

confirming this prediction.

Ultimate Explanations

A variety of evolutionary explanations, including the range size hypothesis and the fertility and parental care hypothesis, have been proposed to explain sex differences in cognition. Several alternative explanations also exist. All of the hypotheses predict sex differences, albeit for different reasons (Jones & Healy, 2006). These hypotheses also vary in the degree of logical or empirical support. The dispersal hypothesis proposes that the sex that disperses farthest will have superior spatial ability (Jones et al. 2003). However, dispersal only occurs once in a lifetime. Thus, spatial demands would not generally differ between dispersers and non-dispersers. Another hypothesis with similar logical problems is the male warfare hypothesis (Geary, 1995). This proposes that males have improved spatial skills as a result of male-male warfare. However, warfare does not occur frequently, and this hypothesis is further limited because it is only applicable to humans (Jones et al., 2003). Another proposal is that the division of labor between males and females has led to differential spatial ability (Silverman et al., 2000). Females are hypothesized to have superior spatial memory for object-locations, a skill that would have benefited gathering. Males are hypothesized to have superior spatial skills in tasks associated with hunting (Geary, 1995; Silverman, Choi, & Peters, 2007). While there is some empirical support for the division of labor hypothesis (Silverman et al., 2000; Silverman et al., 2007), it is only testable in humans and so the predictive value is limited given the generality of sex differences (Jones et al., 2003).

The current study supports the range size hypothesis, although this does not necessarily exclude the fertility and parental care hypothesis as a potential evolutionary mechanism. The pressures describe by each hypothesis may act together to produce the overall differences observed. However, it is important to provide clear support for or against the various hypotheses to the greatest extent possible. By focusing on

explanations that have the most empirical support, and distinguishing between when their effects would be the greatest, we can begin to integrate across levels of explanation.

Integrating Across Levels of Explanation

Although proximate and ultimate levels of explanation are not mutually exclusive, they are often investigated separately. For example, behavioral ecologists have focused on functional questions, evolutionary biologists have focused on phylogenetic questions, psychologists and ethologists have focused on mechanistic questions, and developmental biologists have focused on ontogenetic questions (Healy & Jones, 2002). These levels of explanation provide a useful framework for understanding sex differences in spatial ability (Healy & Jones, 2002). Presently, the finding of sex differences has received empirical investigation and theoretical speculation at each of these levels, but with little integration or overlap (Healy & Jones, 2002). Integrating across levels may be necessary to fully understand the cause of sex differences in spatial ability.

By first establishing a clear understanding of ultimate factors, we can begin to investigate proximate factors. For example, if the selective pressures described by the range size hypothesis are most accurate, we should begin to seek out evidence that testosterone influences spatial ability specifically during the period of range expansion. Several lines of research could address this relationship. For polygynous or promiscuous species, do males experience an increase in testosterone during range expansion? Is this elevation limited to sexually mature males? Do higher testosterone levels (relative increase compared to one's own baseline) correlate to larger range sizes? Or more locomotion? Or increase spatial ability? In species that do not exhibit sexually dimorphic range sizes, are these patterns absent? Are increases in testosterone *not* linked to increased range size or spatial ability? On the contrary, in order to integrate across proximate mechanisms and the fertility and parental care hypothesis, similar measures would need to be considered for the role of estrogen. Does an increase in estrogen during

periods of peak fertility or parental care actually relate to decreased spatial ability? Does this influence reproductive success? Do estrogen levels correlate with performance on spatial tasks?

By addressing these questions, we can be begin to understand the evolutionary pressures that might have shaped sex differences, and uncover the underlying proximate mechanisms, whether biological or experiential, that cause the observed differences.

Benefits of Conducting Cognitive Research in Captivity

Comparative cognition research can be conducted using a variety of theoretical and methodological approaches. Ultimately, these approaches should converge on similar findings. The zoo provides a unique and ideal forum for integrating across fields and levels of explanation.

Cognitive psychology and cognitive ecology have co-existed in parallel for many years, with little interaction (Healy & Jones, 2002). Cognitive psychology focuses on many areas such as learning, memory, perception and use highly controlled procedures to rigorously test various predictions. By controlling as many factors as possible, one can specifically examine the effect of a particular variable. This can provide great detail on the proximate causes of behavior (e.g., hormonal, neurological), but often precludes any speculation regarding the ultimate causes. On the other hand, cognitive ecology is focused on ultimate explanations, in particular how different cognitive abilities have evolved to function in the natural environment. The underlying assumption of this field is that the process of natural selection shapes cognitive abilities in a similar manner to shaping behavioral or morphological characteristics. Thus the lack of overlap between cognitive psychology and ecology may arise due to the differing levels of explanation the fields are interested in pursuing. However, there has been a recent surge of interest in integrating across different levels of explanation. For example, “neuroecology”

specifically focuses on how functioning of the brain is affected by natural selection (Sherry, 2006).

There are some problems in integrating across these fields of research and levels of explanation. A primary problem is that uncovering mechanistic explanations requires the strict control of the environment, and uncovering ultimate explanations often relies on the variability of the natural environment. One could attempt to overcome these differences by collecting specimens from the wild environment (e.g. Gaulin & Fitzgerald, 1986). One might also attempt to study cognition in the wild. This endeavor is plagued by several problems: individuals need to be identifiable, subjects need to behave normally in the presence of the experimenter, and some degree of control of the environment is necessary. One effort to accomplish this has been a field study of hummingbird cognition (Healy & Jones, 2002). Individuals are tagged for identification purposes and responses to artificial feeders can be observed. Variation in the feeders (e.g. interval schedules of nectar availability) can be controlled and any differences across sub-species can be observed. However, this case is somewhat unique.

Typically, attempts to unite the laboratory and the wild are very problematic. However, there is a place in which this research can be conducted: the zoo. Zoos provide an incredibly unique, yet somewhat undertapped, resource for investigating cognitive phenomena. Cognitive psychologists and ecologists can converge in this arena to drive forward our understanding of cognition. There are a number of reasons that zoos provide an ideal situation for cognitive researchers. In a zoo setting, the control required by experimental design is possible. Animal managers have strict control over many factors of the environment: food, lighting, air circulation, access to conspecifics, etc. More importantly, zoos operate using nature as the model of husbandry. In other words, animals are housed in conditions that approximate the wild conditions for that species. This includes temperature, substrates, types of enrichment, food, and social structure. Thus, the aspects of the natural environment which are often removed in standard

laboratory conditions are maintained. These conditions may be relevant to ultimate levels of explanation (e.g. social structure). Furthermore, recent research suggests that the unenriched conditions of some laboratory settings can have a detrimental effect on the brain structure and functioning of captive animals. Thus, laboratory animals may not provide an accurate measure of cognitive ability. Another benefit of conducting research in a zoo is that subjects are individually identifiable. Even for large groups of animals such as flamingos, techniques such as leg bands are used to identify individuals, a task which would be nearly impossible in the field. Another problem in field research is that subjects need to behave normally in the presence of the experimenter, which requires long periods of habituation. In the zoo setting, animals are habituated to human presence and behavior is not influenced as much by this factor as in the wild. Another important benefit to conducting cognitive research in the zoo is the access to a wide variety of species. Thus, evolutionary questions about cognitive abilities can be easily addressed.

Cognitive research in a zoo may also benefit the animals. In general, studies provide cognitive stimulation and require novel responses, which are important forms of enrichment in the zoo (Young, 2003). Not only is the provision of enrichment important for animal welfare, but as previously discussed, there is also evidence to suggest that enrichment improves learning ability and cognitive functioning (Young, 2003). Thus carrying out cognitive research on a regular basis in zoos can improve animal welfare and potentially allow for more accurate measurement of cognitive abilities.

Conclusions

The current research provides strong support for the range size hypothesis in the order Carnivora and helps progress evolutionary theory regarding sex differences in spatial cognition. Furthermore, this work illustrates how conducting cognitive research at zoological institutions is beneficial to all involved. It can allow for more precise

explanations of behavior that integrate across levels, improve our understanding of comparative cognition, and benefit animal populations.

APPENDIX A

SPATIAL MEMORY TASK DATA SHEET

Subject:_____ Sex: M F Cloud cover 0 25 50 75 100% Obs:_____
Time:_____ Date:_____ Temp:____ Location:_____ Baited Feeders:_____

Visit	Feeder visited	Time
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		

Visit	Feeder visited	Time
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		

Notes:

APPENDIX B

ADULT GIANT PANDA BEHAVIOR ETHOGRAM

Behavior	Code	Sampling Method	Definition
Handstand Mark	HM	AO	Animal elevates hind-quarters vertically, front legs support weight; both hind-feet must leave the ground. Usually seen in males.
Leg-Cock Mark	LM	AO	Animal rubs anogenital region with tail up against object or substrate with one hind-leg raised. More common in males.
Reverse Mark	RM	AO	Animal backs into vertical surface before marking.
Squat mark	SM	AO	Animal rubs anogenital region with tail up against object or substrate in squatting posture. More common in females.
Handstand Urine Mark)	UR-HM	AO	Like handstand mark, but instead of rubbing anogenital region, the animal just urinates. If the animal uses this posture to rub the anogenital gland and urinate, score as HM and UR(HM). Only recorded for males.
Leg-Cock Urine Mark	UR-LM	AO	Like leg-cock mark, but instead of rubbing anogenital region, the animal just urinates. If the animal uses this posture to rub the anogenital gland and urinate, score as LM and UR(LM). Only recorded for males.
Olfactory investigate	OI	AO	Places nose close to a substrate and sniffs and/or appears attentive for >1 sec. Sniffing the air or bamboo is not included. If smelling another animal, mark as OI ag/bd
Open Mouth	OM	AO	Animal raises lips, exposing teeth and/or opens mouth while investigating scent. Only recorded for males.
Urinate	UR	AO	Animal voids urine.
Door Directed	DD	I	Animal manipulates closed door with paws, pushes against door with head, sniffs at crack in door, or peers through window in door. Resting or sitting quietly at a door is scored as RS or SA.

Feed/Drink	FD	I	Animal is processing or consuming food. This includes manipulating bamboo for feeding (e.g., stripping leaves, breaking culms, peeling culms). Specify food type being fed on with the following codes: **B** - bamboo; **O** – any other food; also scored if animal drinks water.
Locomote	LC	I	Short bout of directional travel, fast or slow, between two points, includes pacing and climbing.
Maintenance	MA	I	Scratching body with paws, licking and/or biting fur, or rubbing a small part of its body repetitively back and forth against an object, as if to scratch an itch.
Rest	RS	I	Animal is relaxed in a lying or sitting position, either awake or asleep.

Stationary Alert	SA	I	Alert, standing, sitting or lying quietly, but remaining attentive, moving head from side to side and/or sniffing air, perhaps attending to external stimuli. Simply opening the eyes and/or shifting position while resting does not apply.
Elimination	EL	I	Animal voids feces or urine.
Other Solitary	OSOL	I	Animal performs any solitary behavior that does not involve another animal which does not fit any of the other definitions on the ethogram.
Social	SOC	I	Animal performs any behavior involving another animal through a barrier. For example Lun moans at MeiLan through howdy on the beep. Record MO and make a check in the SOC column. Describe behavior in Notes section.
Stereotypic	STE	I	Animal performs any repetitive stereoptyped behavior (e.g., pacing – traveling same path at least 3 times, head toss, pirouette, etc.). Includes self sucking (SUC) that occurs on the beep – also make a tick mark in the appropriate section if SUC occurs on the beep, so that frequency is recorded.
Not Visible	NV	I	It is not possible to see what the animal is doing.
Bleat	BL	OZ	A twittering, goat-like call of variable length (1-3s). Contact call, appeasing, non-aggressive, affiliative.
Chirp	CH	OZ	Short, tonal, high-pitched, descending in pitch toward end. Most common during estrus. Affiliative, promotes social proximity.
Bark	BA	OZ	Short (0.1-0.3s), fairly noisy, similar to dog bark. Threat, causes withdrawal by receiving animal.
Moan	MO	OZ	Low-pitched, low to medium amplitude call of variable duration. Often has several short starting elements. Often grades with barks, chirps, and bleats. Mild threat, often used by female to discourage approach by male.
Growl	GR	OZ	Long, noisy low-pitched growl similar to a dog's. Aggressive, often accompanies or precedes fighting. Only used by attacking animal.
Honk	HK	OZ	Short (< 0.5s), tonal, low-pitched, nasal, falling in pitch, produced repetitively in a series. Often used when distressed or frustrated.
Roar	RO	OZ	Very loud, intense, harsh vocalization (highest level threat).
Chomp	CM	OZ	Alternate rapid opening and closing of mouth, with teeth coming together audibly. Defensive (e.g., when the female avoids the male's approach), mild threat.
Huff	HU	OZ	Audible expulsion of air through open mouth (anxious, mild threat).
Snort	SN	OZ	More intense expulsion of air through nose (threat, apprehension).
Squeal	SQ	OZ	Short, high-pitched, open-mouthed call. Often used by subordinate animal in a fight or in response to pain.
Whine	WH	OZ	High-pitched, medium intensity, often repetitive vocalization, usually occurs when: animals are wrestling, an animal is having behavior directed toward it, but it is engaged in another activity (e.g., climbing, resting).

APPENDIX C

ADULT GIANT PANDA BEHAVIOR DATA SHEET

Date _____ Focal _____ Cloud 0% 25% 50% 75% 100%
Time _____ Food Pres _____ Enclosure _____
Temp _____ Humidity _____ Observer _____
Influence _____

	Instantaneous											One-Zero
Min	DD	FD	LC	MA	RS	SA	EL	OSOL	SOC	STE	NV	Vocaliz.
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
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17												
18												
19												
20												
21												
22												
23												
24												
25												
26												
27												
28												
29												
30												
Total	DD	FD B O	LC	MA	RS	SA	EL	OSOL	SOC	STE	NV	Voc.

All-Occurrence

Behav	# Occurr	Total	Behav	# Occurr	Total
HM					
LM			UR		
OI			UR-HM		
OM			UR-LM		
RM					
SM					

Notes:

APPENDIX D

ADULT OTTER BEHAVIOR ETHOGRAM

Behavior	Code	Sampling Method	Definition
Locomote	LC	I	Short bout of directional travel, fast or slow, between two points, includes pacing and climbing.
Maintenance	MA	I	Scratching body with paws, licking and/or biting fur, or rubbing a small part of its body repetitively back and forth against an object, as if to scratch an itch.
Rest	RS	I	Animal is relaxed in a lying or sitting position, either awake or asleep.
Feed/Drink	FD	I	Animal consumes food or water.
Foraging	FO	I	Animal manipulates or searches for food, but is not actively consuming.
Elimination	EL	I	Animal voids feces or urine.
Other Solitary	OSOL	I	Animal performs any solitary behavior that does not involve another animal which does not fit any of the other definitions on the ethogram.
Other Social	OSOC	I	Animal performs any behavior involving another animal which does not fit any of the other definitions on the ethogram.
Stereotypic	STE	I	Animal performs any repetitive stereoptyped behavior.
Not Visible	NV	I	It is not possible to see what the animal is doing.
General Interaction	GI	I	Two otters are eating, running, standing or lying together.
Olfactory investigate	OI	AO	Places nose close to a substrate and sniffs and/or appears attentive for >1 sec.
Urinate	UR	AO	Animal voids urine.
Defecate	DE	AO	Animal voids feces.
Grooming	GR	AO	Two animals engage in gnawing or touching fur of other individual.
Non-contact playing	NCP	AO	Two animals chase one another for at least one body length.
Contact playing	CP	AO	Two animals wrestle or roll together.
Mounting	MT	AO	One animal mounts another.
Fighting	FT	AO	Two animals bite or screech while in physical contact.
Screaming	SC	AO	Animal screams at another individual.
Vocalization	VC	OZ	Animal vocalizes.

APPENDIX E

ADULT OTTER BEHAVIOR DATA SHEET

Date _____ Focal _____ Cloud 0% 25% 50% 75% 100%
Time _____ Food Pres _____ Enclosure _____
Temp _____ Humidity _____ Observer _____
Influence _____

Instantaneous **One-Zero**

Min	LC	MA	RS	FD	FO	EL	OSOL	OSOC	STE	GI	NV	Vocaliz.
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
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21												
22												
23												
24												
25												
26												
27												
28												
29												
30												
Total	LC	MA	RS	FD	FO	EL	OSOL	OSOC	STE	GI	NV	Voc.

All-Occurrence

Behav	# Occurr	Total	Behav	# Occurr	Total
OI					
UR			MT		
DE			FT		
GR			SC		
NCP					
CP					

NOTES:

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